HYPERBRANCHED POLYMERS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority from U.S. Provisional Patent Application No. 60/540,374, filed on January 30, 2004, the contents of which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

The invention relates to polymeric compositions, and methods of making the same.

BACKGROUND

The concept of highly branched polymers (also known as hyperbranched polymers) was initially introduced by Flory fifty years ago (see, for example, Flory, P. J. *Principles of Polymer Chemistry*; Cornell University Press: Ithaca, 1953). Hyperbranched polymers are further described by Frechet et al., *J. Macromol. Sci.-Pure Appl. Chem.*, 1996, *A33*, 1399, Kim et al., *J. Am. Chem. Soc.*, 1990, *112*, 4592, Leduc et al., *J. Am. Chem. Soc.*, 1996, *118*, 11111, Frechet and Henmi et al., *Science*, 1995, *269*, 1080, Malkoch et al., *Macromolecules*, 2002, *35*, 8307, Magnusson et al, *Macromolecules*, 2000, *33*, 3099, and Ihre et al., *Macromolecules*, 1998, *31*, 4061. Hyperbranched polymers are typically synthesized by a one-step polymerization process, which can yield low molecular weight products with broad molecular weight distributions (polydispersity). Upon further modification, the molecular weight of these polymers can be increased, but their architecture typically only approximates a perfect dendrimer (i.e., a "pseudo-dendrimer," see, for example Haag et al., *J. Am. Chem. Soc.*, 2000, *122*, 2954).

Dendrimers with a nearly perfect regular structure that have precise molecular weights have been synthesized by iterative synthetic steps. Such dendrimers have been described by Bosman et al., *Chem. Rev.* 1999, *99*, 1665, Fischer et al., *Angew. Chem.-Int. Edit.*, 1999, *38*, 885, Newkome et al., *Dendritic Molecules: Concepts, Syntheses, Perspectives*; VCH: Weinheim ; New York, 1996 and Jayaraman et al., *J. Am. Chem. Soc.*, 1998, *120*, 12996.

Hyperbranched polymers typically exhibit a compact, globular structure in combination with an exceptionally high number of terminal groups that can be further functionalized. Due to their inert building blocks and multiple chain termini, hyperbranched polymers can be used for various applications, for example, as

multifunctional dendritic macromolecular supports for applications ranging from drug- or catalyst- carriers to highly functional cross-linkers, surface coating components, photoactive materials, etc. In addition, the water solubility, biocompatibility, and biodegradability of hydrophilic hyperbranched polymers make them useful for different medical applications, for example, gene delivery. Using hyperbranched polymers for gene delivery is described by Stiriba et al., *Angew. Chem.-Int. Edit.*, 2002, *41*, 1329 and Haensler et al., *Bioconjugate Chem.*, 1993, *4*, 372.

Recent disclosures in non-viral gene delivery have revealed that branched polyethyleneimine (BPEI) and dendrimers therefrom are effective as carriers of exogenous genetic material (see, for example, Boussif et al., *Proc. Natl. Acad. Sci. U.S.A.*, 1995, *92*, 7297). BPEI alone, however, can be toxic and can form unstable DNA complexes. BPEI and dendrimers modified with poly(ethylene glycol) derivatives showed less toxicity than their counterparts, but have less transfection efficiency, e.g., as described by Choi et al., *J. Am. Chem. Soc.*, 2000, *122*, 474, Luo et al., *Macromolecules*, 2002, *35*, 3456 and Bronich et al., *Langmuir*, 1998, *14*, 6101. To modify these polymers, the amino groups are typically reacted with poly(ethylene glycol) derivatives to decrease cytotoxicity and to enhance stability of DNA-dendrimer complexes. Although the DNA-dendrimer complexes tend to be more stable, PEG-modification usually results in larger complex particles that can have substantially lower transfection efficiency when compared to un-modified BPEI.

An alternative approach to reduce cytotoxicity is to make a block or a graft co-polymer of PEG and a polycation. This approach has been described by Leduc, et al., *J. Am. Chem. Soc.* 1996, *118*, 11111, Frechet, et al., *Science*, 1995, *269*, 1080, Malkoch et al., *Macromolecules*, 2002, *35*, 8307 and Magnusson, et al., *Macromolecules*, 2000, *33*, 3099. Work by Bogdanov, et al., *Radiology*, 1993, *187*, 701 and Weissleder, et al., *Nat. Biotechnol.*, 1999, *17*, 375, for example, has shown that PEG-grafted poly (L-lysine) has shown a long blood circulation time *in- vivo*, has a low cytotoxicity, and is suitable for blood pool imaging *in-vivo*. These polymers, however, yield typically large complexes with DNA that have efficient shielding of a positive charge of the core polycation by PEG chains. As a result, many of these polymers are often not suitable for *in-vivo* gene delivery (see, for example, Nguyen, et al., *Gene Ther.*, 2000, *7*, 126).

Star polyethylene oxide (STARPEO) has been used for various biomedical applications, including tissue engineering. However, STARPEO can be expensive to

produce since it is typically prepared by anionic polymerization methods (see, for example, Rein et al., *Acta Polym.*, 1993, *44*, 225).

SUMMARY

This invention is based, in part, on the discovery of new hyperbranched polymers, e.g., soluble hyperbranched polymers, that include an inner core and an outer shell, and new methods of synthesis. The inner core includes a network of bound molecular chains that can include ether linkages and may contain, for example, a plurality of core hydroxyl groups. The outer shell may include, for example, hydroxyl groups bound to an outer portion of the core. The hyperbranched polymers disclosed herein have many useful applications, including the delivery of therapeutic agents, imaging agents, or sensors to the bodies of animals, such as mammals, or of humans. These polymers are also useful as hydrogels in tissue engineering and for the preparation of synthetic organs. Non-biological applications of the polymers disclosed herein include solid polymer electrolytes and additives for paints, for example, water-based paints. The hyperbranched polymers may be non-degradable or they may be biodegradable. For example, a portion of the hyperbranched polymer may be degradable or cleavable.

In one aspect, the invention features hyperbranched polymers, e.g., soluble hyperbranched polymers, that include an inner core and an outer shell. The outer shell includes active hydrogen-containing groups bound to an outer portion of the core. The inner core includes a network of bound molecular chains that include heteroatoms. At least one of the chains includes at least one moiety that includes two heteroatoms spaced apart by at least two atoms. The heteroatoms can be, for example, oxygen atoms, sulphur atoms, nitrogen atoms, or mixtures thereof. The two heteroatoms can be spaced apart by, for example, two, four, six, eight, or more atoms, e.g., twelve atoms. The active hydrogen containing groups can be, for example, hydroxyl groups, thiol groups, primary amine groups, secondary amine groups, and mixtures thereof. The moiety can be, for example, $-OCH_2C(CH_2OH)(H)O-$, $-OCH_2C(OH)(OH)CH_2O-$, $-OCH_2C(CH_2(P_1))(CH_2(P_2))CH_2O-$, $-OCH_2C(OH)(OH)O-$, $-OC(H)(P_3)CH_2O-$, $-OCH_2C(OP_4)(H)CH_2O-$, $-OCH_2C(OH)(H)CH_2O-$, in which P_1, P_2, P_3, and P_4 are, e.g., branched glycol polymers, or mixtures thereof.

In another aspect, the invention features methods of making hyperbranched polymers, e.g., a soluble hyperbranched polymer. The methods include obtaining a first component including at least one epoxide group and obtaining a second component

including at least one active hydrogen group. The first and second components are combined in the presence of an electrophilic initiator and the components are allowed to react for a time and under conditions sufficient to produce a hyperbranched polymer. The total number of epoxide groups plus the total number of active hydrogen groups is four or greater, e.g., five, six, seven, or more, e.g., 10. The first component can be, for example, glycidol, ethylene glycol diglycidyl ether, glycerol diglycidyl ether, glycerol triglycidyl ether, tetraglycidyl pentaerythritol, polyethylene glycol diglycidyl ether, glycerol polyethylene glycol triglycidyl ether, tetraglycidyl polyethylene glycol erythritol, or mixtures thereof. The second component can be, for example, glycerol, erythritol, pentaerythritol, polycaprolactone triol, glycerol ethoxylate triol, pentaerithrytol ethoxylate, or mixtures thereof. These methods allow for, in part, the preparation of soluble, highly branched polymers that can be functionalized in subsequent steps.

In another aspect, the invention features hyperbranched polymers, e.g., soluble hyperbranched polymers. The hyperbranched polymers include an inner core, and an outer shell that includes hydroxyl groups bound to an outer portion of the core. The inner core includes a network of bound molecular chains including ether linkages. At least one of the chains includes at least one moiety that includes two oxygen atoms spaced apart by at least four atoms, e.g., carbon atoms. In some embodiments, the moiety is $-O(CH_2)_nO-$, in which n is an integer from 4 to 12, or mixtures thereof.

In certain embodiments, the hyperbranched polymers described herein can have a number average molecular weight, measured against monodisperse standards of polyethylene glycol (PEG), of from about 5,000 Daltons to about to 500,000 Daltons, e.g., from about 50,000 to about 250,000, or about 100,000 to about 200,000 and/or a hydrodynamic diameter, measured in aqueous solution, e.g., that is from about 15 to about 2000 nm, e.g., 100 nm to about 1000 nm.

In some embodiments, the total number of core and shell hydroxyl groups is from about 75 to about 1000.

The hyperbranched polymers can have at least portions of the outer shell that are functionalized with an activated component, e.g., an acyl chloride group, and the hyperbranched polymer can further include a moiety, e.g., a polymer, grafted onto a portion of the activated component. Suitable polymers include, e.g., polymers containing unsaturation, e.g., a carbon-carbon double bond, polyoxazolines, polyesters, polycarbonates, polyamides, or mixtures thereof.

In another aspect, the hyperbranched polymers, e.g., soluble hyperbranched polymers, include an inner core including a network of bound molecular chains including ether linkages and core hydroxyl groups, and an outer shell comprising hydroxyl groups bound to an outer portion of the core. The hyperbranched polymers can be formed by a reaction of a first component including at least two epoxide groups and a second component including at least two hydroxyl groups, together with an electrophile, followed by quenching.

The hyperbranched polymer can, e.g., include portions that are formed by a reaction that further includes a cyclic ether, e.g., tetrahydrofuran, the cyclic ether becoming structurally a part of the hyperbranched polymer.

In some embodiments, at least a portion of the outer shell is functionalized with an activated component, the activated component enhancing the reactivity of the hyperbranched polymer towards addition, substitution, e.g., electrophilic substitution, or elimination reactions.

The hyperbranched polymers can further include, e.g., a second polymer grafted onto at least some of the activated components in the outer shell. Suitable second polymers include, e.g., polymers containing unsaturation, polyoxazolines, polyesters, polycarbonates, polyamides, or mixtures thereof. In some implementations, the second polymer is prepared by electrophilic polymerization of an oxazoline, e.g., a 2-ethyl(alkyl)-2-oxazoline, or methyl-2-oxazoline. If an oxazoline polymer is used, it can have a hydrodynamic diameter, measured in water as solvent, of e.g., from about 500 nm to about 3000 nm, e.g., 750, 1000, 1500, or 2000 nm. In other implementations, the second polymer is prepared from electrophilic polymerization of 2-(dimethylamino)ethyl methacrylate.

In another aspect, the invention features methods of making hyperbranched polymers, e.g., soluble hyperbranched polymers. The method includes obtaining a first component including at least two epoxide groups, and obtaining a second component including at least two hydroxyl groups. The first and second components are reacted in the presence of an electrophilic initiator for a time and under conditions sufficient to produce a hyperbranched polymer.

In some embodiments, conditions include employing a diluent, e.g., a halogenated diluent, e.g., methylene chloride, or a cyclic ether, e.g., tetrahydrofuran (THF). The cyclic ether can participate in the reaction, e.g., undergoing a ring-opening reaction, and

becoming part of the hyperbranched polymer. Conditions can also include, e.g., maintaining a temperature of less than 10 °C.

The first component can be, e.g., a small molecule with a molecular weight of less than 750 Daltons, and/or the second component can be, e.g., a small molecule with a molecular weight of less than 750 Daltons.

In some embodiments, the first component includes, e.g., two epoxide groups disposed at ends of the first component, e.g., glycerol dicylcidyl ether, polyethylene glycol diglycidyl ether, or mixtures thereof, and the second component includes, e.g., four hydroxyl groups disposed at the ends of the second component. In some implementations, a ratio of the first component to the second component is less than about 1, e.g., 0.75 or 0.5.

The electrophilic agent, e.g., a Lewis acid, can be employed, e.g., at a concentration from about 0.001 to about 0.1 mole/L, e.g., 0.05, 0.025, 0.01, or 0.005 mol/L. For example, when a Lewis acid is employed, the Lewis acid can be, e.g., boron trifluoride, tin chloride, mixtures thereof, or equivalents thereof. For example, boron trifluoride can be in the form of an etherate, e.g., boron trifluoride diethyl etherate.

The methods can further include quenching the reaction to halt polymerization.

In some embodiments, the methods further include activating at least some hydroxyl groups of the resulting hyperbranched polymers by reacting the hyperbranched polymers with an activating component, the activating component enhancing the reactivity of the hyperbranched polymer towards substitution and elimination reactions, e.g., electrophilic substitution reactions. Suitable activating components include, e.g., strong organic acids, e.g., trifluoroacetic acid, strong mineral acids, alkoxides, hydrides, active metals, or compatible mixtures thereof. In some implementations, the activating component becomes bound to an oxygen of a hydroxyl group. In such instances, the activating component includes an acyl group, e.g., an active acyl group, e.g., an acyl chloride. The activating group can include other groups, e.g., sulfonyl groups, e.g., sulfonyl chlorides.

In another aspect, the invention features methods of making hyperbranched polymers, e.g., soluble hyperbranched polymers. The methods include obtaining a first component that includes at least one epoxide group, and obtaining a second component that includes at least one hydroxyl group. The first and second components are combined in the presence of an electrophilic initiator, and allowed to react for a time and under conditions to produce a hyperbranched polymer. At least one of the components has a

total polymerizable functionality of 3 or greater, e.g., 4, 5, 6, or more, e.g., 8. In some embodiments, the second component is, e.g., $(R_4CH_2)(R_3CH_2)(R_2CH_2)(R_1CH_2)C$, in which R_1-R_4 are each the same or different and selected from $(ACH_2CH_2)_nAH$, where A is an oxygen or a sulphur atom, and n is greater than or equal to 1, e.g., 2, 3, 5, 10, 100, 500 or 1000. For example, n can be between about 2 and about 200, e.g., 5, 10, 25, 50, 75, 100, 125, 150, or 175. For example, the second component can be $(HOCH_2)_4C$. In other embodiments, the second component is $(R_4CH_2)(R_3CH_2)(R_2CH_2)(R_1CH_2)C$, in which R_1-R_4 are each the same or different and are selected from $(N(R_5)CH_2CH_2)_nAH$, where A is an oxygen or a sulphur atom, or NR_6; where R_5 and R_6 are each the same or different and can be H, or a straight, branched, or substituted alkyl, alkenyl, or aryl moiety, and n is greater than or equal to 1, e.g., 2, 3, 5, 10, 100, 500, or 1000.

In some specific embodiments, the second component can be $(OH)C(CH_2OH)(CH_2OH)R_1$, in which R_1 is a straight, branched or substituted alkyl, alkenyl, or aryl moiety, and n is greater than or equal to 1, and the second component is $R_1(OR_2)(OR_3)C(OR_4)$, in which R_1 is a straight, branched, or substituted alkyl, alkenyl, or aryl moiety, in which R_2-R_4 are each the same or different and include a hydroxyl-containing polymer, e.g., a polymeric moiety that includes between about two to about four hydroxyl groups, e.g., a polymer in which the hydroxyl is disposed at the end of the polymer. For example, the polymer can be, e.g., a polycaprolactone.

In some embodiments, the first component is $ACH_2(OCH_2CH_2)_nOCH_2A$, in which A is a moiety that includes an epoxide, or a substituted epoxide group, and n is greater than or equal to 1, e.g., 2, 3, 4, 10, 20, 50, 100, 1000, or more, e.g., 10,000. In some implementations, the first component is polymeric moiety including at least one epoxide group.

Adducts of any of the above described polymers can be formed with nucleic acids, therapeutic agents, and/or imaging agents. Alternatively, adducts between any of the above described polymers and components carrying such nucleic acids, therapeutic agents, and/or imaging agents can also be formed.

Advantages of the hyperbranched polymers include a highly adaptable, readily functionalizable architecture system that can be tailored for medical and non-medical applications. The size of the polymers can be readily modified, for example, for delivering therapeutic agents, for example, nucleic acids. The size of the polymers can be increased, for example, by adding material or by changing the charge on the chains. The hydrophilicity of the polymers is readily adaptable, for example, by modification of the

inner core or outer shell. For example, the hydrophilicity of the inner core can be modified by increasing the number of atoms, e.g., carbon atoms, between the heteroatoms of the moiety, for example, oxygen atoms. Advantages also include relatively low toxicity, which allows the disclosed hyperbranched polymers to be used *in vivo*.

Advantages to the methods disclosed herein include high yields, easy purification, and relatively low cost manufacturing methods that can be easily scaled up or down.

"Activating components" as defined herein are moieties that enhance the reactivity of a group, for example, a hydroxyl group, towards substitution, addition, and/or elimination reactions relative to the unactivated group as measured by relative rate constants. For example, protonation or chelation of a hydroxyl group makes it more susceptible to substitution reactions relative to the unactivated hydroxyl group.

A small molecule is a monomeric or an oligomeric moiety with a formula weight or number average molecular weight, measured by gel-permeation chromatography (GPC) against monodisperse standards of polyethylene glycol (PEG), of less than about 750. For example, ethylene glycol is a small molecule, as is polyethylene glycol with a number average molecular weight of less than about 750 Daltons. A large molecule is a monomeric, oligomeric, or polymeric moiety with a formula weight or number average molecular weight of greater than about 750 Daltons. For example, the C_{60} molecule functionalized with a single carboxylic acid group (C_{60}-COOH, formula weight, FW = 766 daltons) is a large molecule, as is a protein with a number average molecular weight of about 50,000.

A "heteroatom" is an atom selected from the group including nitrogen, sulphur, oxygen, or mixtures thereof.

A "polymerizable functionality" is a group capable of incorporating into a polymer through covalent bonds. Examples include epoxy groups and hydroxyl groups.

The term "alkyl" denotes straight chain, branched, mono- or poly-cyclic alkyl moieties. Examples of straight chain and branched alkyls include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, t-butyl, amyl, isoamyl, sec-amyl, 1,2-dimethylpropyl, 1,1-dimethylpropyl, pentyl, hexyl, 4-methylpentyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 1,1-dimethylbutyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 1,2,2-trimethylpropyl, 1,1,2-trimethylpropyl, heptyl, 5-methylhexyl, 1-methylhexyl, 2,2-dimethylpentyl, 3,3-dimethylpentyl, 4,4-dimethylpentyl, 1,2-dimethylpentyl, 1,3-dimethylpentyl, 1,4-dimethylpentyl, 1,2,3-trimethylbutyl, 1,1,2-trimethylbutyl, 1,1,3-trimethylbutyl, octyl, 6-methylheptyl, 1-

methylheptyl, 1,1,3,3-tetramethylbutyl, nonyl, 1-, 2-, 3-, 4-, 5-, 6- or 7-methyloctyl, 1-, 2-, 3-, 4- or 5-ethylheptyl 1-, 2- or 3-propylhexyl, decyl, 1-, 2-, 3-, 4-, 5-, 6-, 7- and 8-methylnonyl, 1-, 2-, 3-, 4-, 5- or 6-ethyloctyl, 1-, 2-, 3- or 4-propylheptyl, undecyl 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8- or 9-methyldecyl, 1-, 2-, 3-, 4-, 5-, 6- or 7-ethylnonyl, 1-, 2-, 3-, 4- or 5-propyloctyl, 1-, 2- or 3-butylheptyl, 1-pentylhexyl, dodecyl, 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9- or 10-methylundecyl, 1-, 2-, 3-, 4-, 5-, 6-, 7- or 8-ethyldecyl, 1-, 2-, 3-, 4-, 5- or 6-propylnonyl, 1-, 2-, 3- or 4-butyloctyl, 1,2-pentylheptyl and the like. Examples of cyclic alkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl, and the like.

The term "alkenyl" denotes straight chain, branched, mono- or poly-cyclic alkene moieties, including mono- or poly-unsaturated alkyl, or cycloalkyl groups. Examples of alkenyl groups include vinyl, allyl, 1-methylvinyl, butenyl, iso-butenyl, 3-methyl-2-butenyl, 1-pentenyl, cyclopentenyl, 1-methylcyclopentenyl, 1-hexenyl, 3-hexenyl, cyclohexenyl, 1-heptenyl, 3- heptenyl, 1-octenyl, cyclooctenyl, 1-nonenyl, 2-nonenyl, 3-nonenyl, 1-decenyl, 3-decenyl, 1,3-butadienyl, 1,4-pentadienyl, 1,3-cyclopentadienyl, 1,3-hexadienyl, 1,4-hexadienyl, 1,3-cyclohexadienyl, 1,4-cyclohexadienyl, 1,3-cycloheptadienyl, 1,3,5-cycloheptatrienyl, 1,3,5,7-cycloocta-tetraenyl, and the like.

The term "aryl" denotes single, polynuclear, conjugated, or fused residues of aromatic hydrocarbons. Examples of aryl include phenyl, biphenyl, phenoxyphenyl, naphthyl, tetahydronaphthyl, anthracenyl, dihydroanthracenyl, benzanthracenyl, dibenzanthracenyl, phenantrenyl, and the like.

A "substituted" group is one that includes additional moieties selected from oxygen, nitrogen, sulphur, alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, hydroxy, alkoxy, alkenyloxy, alkynyloxy, aryloxy, carboxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloalkynyloxy, haloaryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheterocyclyl, azido, amino, alkylamino, alkenylamino, alkynylamino, arylamino, benzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl, acylamino, acyloxy, aldehydo, alkylsulphonyl, arylsulphonyl, alkylsulphonylamino, arylsulphonylamino, alkylsulphonyloxy, arylsulphonyloxy, heterocyclyl, heterocycloxy, heterocyclylamino, haloheterocyclyl, alkylsulphenyl, arylsulphenyl, carboalkoxy, carboaryloxy, mercapto, alkylthio, arylthio, acylthio, and the like.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this

invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematic representation of a hyperbranched polymer including a core and an outer shell according to one embodiment.

Figs. 2-4 are schematic representations of the hyperbranched polymer of Fig. 1 with at least an outer shell being activated.

Fig. 5 is a graph of percentage cytotoxicity vs. polymer concentration for a number of hyperbranched polymers.

Fig. 6 is a graph of transfection vs. polymer concentration for a number of hyperbranched polymers.

Fig. 7 is a schematic representation of a number of applications for the hyperbranched polymers.

Fig. 8 is a graph that shows cytotoxicity profiles of linear polyethyleneimine (LPEI), branched polyethyleneimine (BPEI) and hyperbranched block copolymer; (Table 1): HPGBPEI, HGPL6, HGPL7 in COS-1 cell culture after 48 hours incubation.

Fig. 9A is a graph that shows Luciferase expression in COS 1 cells after a 48 hours transfection in serum-containing medium: transfection efficiency of LPEI, HGPL6, HGPL7, HPGBPEI and BPEI; polymer complex with plasmid was prepared by mixing 1 mg of pCMV-luc with various polymer amounts (shown in x-axis).

Fig. 9B is a graph that shows Percent inhibition of Luciferase expression in Gli36 cells after a 48 hour transfection of siRNA vector in serum-containing medium: inhibition in presence control empty vector (EV) LPEI-EV, HGPL6-EV and Si-RNA against luciferase mRNA vector (Luc) HGPL6-Luc, LPEI-Luc. Polymer complex with plasmid was prepared by mixing 1 μg of pCMV-luc with various polymer amounts (shown in x-axis).

Fig. 10A is a graph that shows percent inhibition of Luciferase expression in Gli36 cells after a 48 hour transfection of siRNA vector in serum-containing medium: inhibition in presence control empty vector (EV) HPGPEI-EV, BPEI-EV and Si-RNA against luciferase mRNA vector (Luc) HPGBPEI-Luc, BPEI-Luc. Polymer complex with plasmid was prepared by mixing 1 μg of pCMV-luc with various polymer amounts (shown in x-axis).

Fig. 10B is a graph that shows Percent inhibition of Luciferase expression (RLU/mg of Protein) in HeLa cells after a 48 hour transfection of siRNA vector in serum-containing medium: Inhibition in presence control empty vector (vector) HPGBPEI, BPEI and Si-RNA against luciferase mRNA vector (RNAi); untreated cells were used as control.

DETAILED DESCRIPTION

The hyperbranched polymers disclosed herein include an inner core and an outer shell. The inner core includes a network of bound molecular chains including heteroatoms, for example, oxygen and sulfur atoms. The inner core can include, for example, covalently bound molecular chains including ether linkages, and the chains can include a moiety that includes two heteroatoms, for example, oxygen atoms, spaced apart by at least two atoms, e.g., 2, 3, 4, 5, 6, or more, e.g., 10. The outer shell can, for example, include hydroxyl groups bound to an outer portion of the core. The moiety can be, for example, $-O(CH_2)_nO-$ where n is an integer greater than or equal to 2, e.g., 2, 3, 4, 5, 6 or more, e.g., 10. Such polymers can be prepared by, for example, by an electrophilic initiated polymerization as will be further discussed below.

Hyperbranched Glycols

Fig. 1 shows a soluble hyperbranched glycol polymer 8 that includes an inner core 10 and an outer shell 12. The inner core includes a network of bound molecular chains including ether linkages. The outer shell includes hydroxyl groups 14 bound to an outer portion of the core. The hyperbranched glycol polymer is formed by a reaction of a first component that includes epoxide groups and a second component that includes hydroxyl groups, together with an electrophile. The polymeric glycol is released upon quenching to halt the polymerization.

Suitable first components include glycidol, ethylene glycol diglycidyl ether, glycerol diglycidyl ether, glycerol triglycidyl ether, tetraglycidyl pentaerythritol,

polyethylene glycol diglycidyl ether, glycerol polyethylene glycol triglycidyl ether, and tetraglycidyl polyethylene glycol erythritol.

Suitable second components include glycerol, erythritol, pentaerythritol, polycaprolactone triol , glycerol ethoxylate triol, and pentaerithrytol ethoxylate.

Suitable electrophilic agents include proton sources, Lewis acids, and other electron deficient moieties, for example, boron trifluoride, tin chloride, and antimony fluoride. A suitable concentration of the electrophilic agent is from about 0.001 mole/L to about 0.1 mole/L, e.g., 0.005, 0.01, 0.025, 0.05, or more, e.g., 0.075 mol/L.

In general, the reaction involves mixing an epoxide, and a polyol with an electron deficient initiator and allowing polymerization to occur in an inert atmosphere, for example, by using argon gas. Typically, the reaction mixture during polymerization is maintained below room temperature. It is believed that the hyperbranched polymer, at least in some cases, grows through a living cationic polymer intermediate. The polymerization is typically quenched with acidic water or methanol when the polymer has reached a desired size or molecular weight. In some embodiments, the solvent incorporates into the polymer.

In some embodiments, reaction conditions include a temperature of between about –70°C and about 15°C, e.g., from about –25°C to about –10°C. In some embodiments, conditions include a diluent. The diluent can be, for example, a halogenated solvent, for example, methylene chloride, or it can be a cyclic ether, for example, tetrahydrofuran. Other suitable cyclic ethers include dioxane.

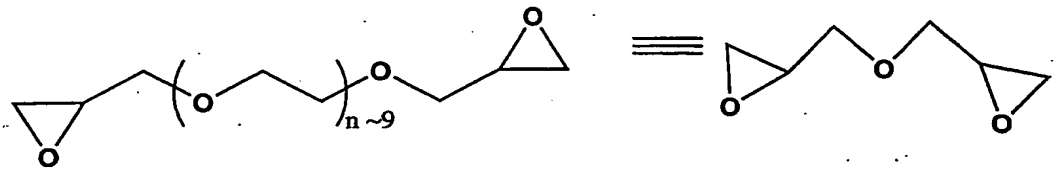
The molecular weight of the polymer depends upon the conditions under which the polymerization is performed. Increasing the initial concentration of the electrophile generally decreases the molecular weight of the resulting polymer. In some cases, the molecular weight depends upon the solvent (if any) used during the polymerization. For example, polymerizations carried out in tetrahydrofuran (THF) yield hyperbranched glycols of higher molecular weight than those prepared in methylene chloride. Lower temperatures generally favor higher molecular weights. In general, the number average molecular weight of the new hyperbranched glycol polymers is from about 10,000 to about 250,000 or more, e.g., 100,000, 150,000, 200,000, 300,000 or more, e.g., 500,000. The polydispersity is from about 1 to about 5, e.g., 1.25, 1.5, 2, 3 or 4.

Depending upon the time allowed and conditions under which the polymerization is carried out, we have found that the hydrodynamic diameter is from about 10 nm to

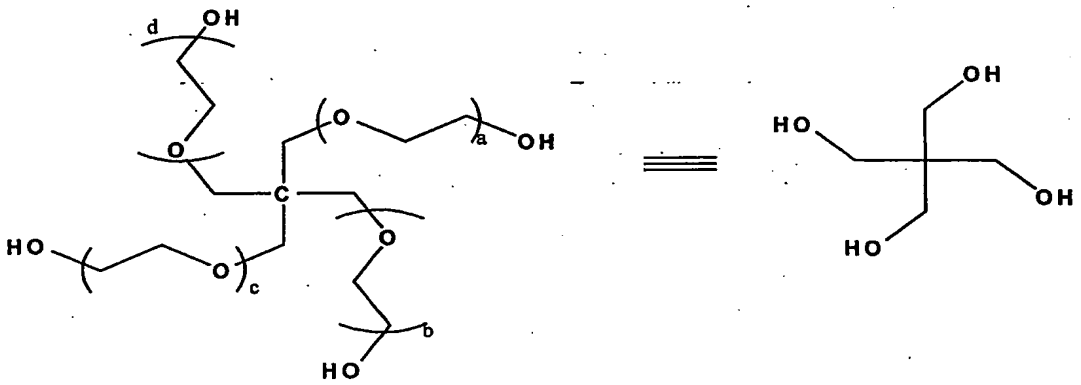
about 2000 nm or more, for example, 15 nm, e.g., 20 nm to 80 nm, 50 nm to 100 nm, or 100 nm to 200 nm or more up to 2000 nm.

Depending upon conditions and first and second components used, the total number of hydroxyl groups is from about 75 to about 1000 or more, for example, 2000, e.g., 100 to 400, or 200 to 800.

In one embodiment, a soluble hyperbranched glycol is prepared by polymerization of a mixture of polyethylene glycol diglycidyl ether (**A**, MW 526) and pentaerythritol ethoxylate (**B**, MW 797) using electrophilic polymerization and THF as diluent or solvent. The structures and simplified structures of each component are shown below.



A



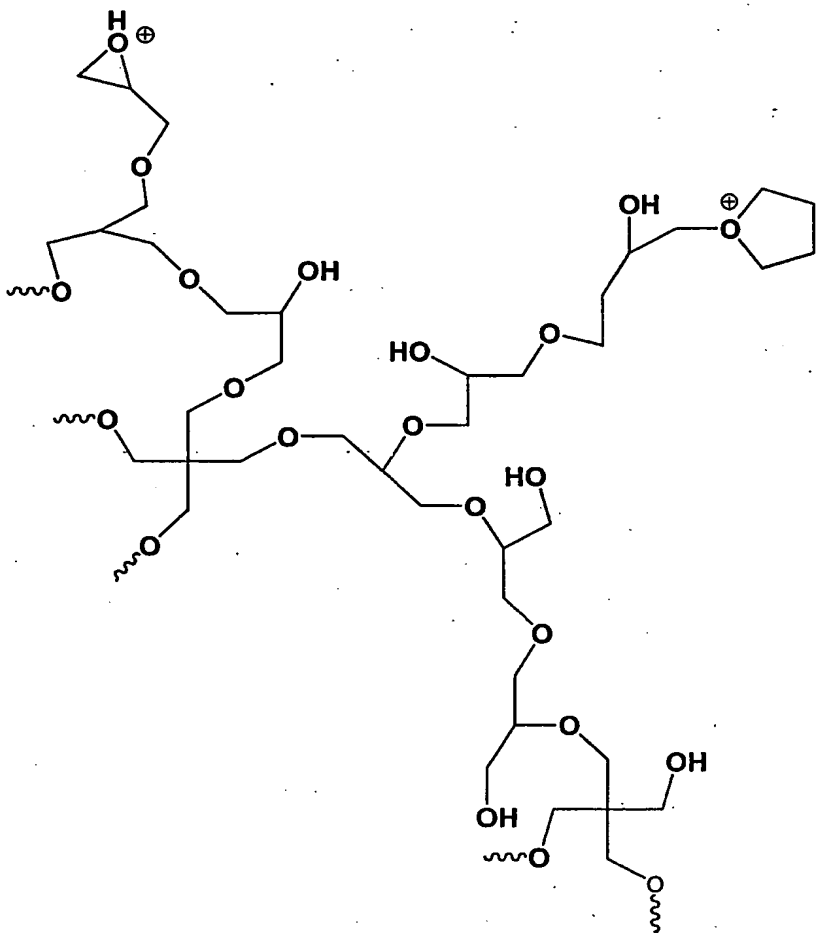
a+b+c+d = 15

B

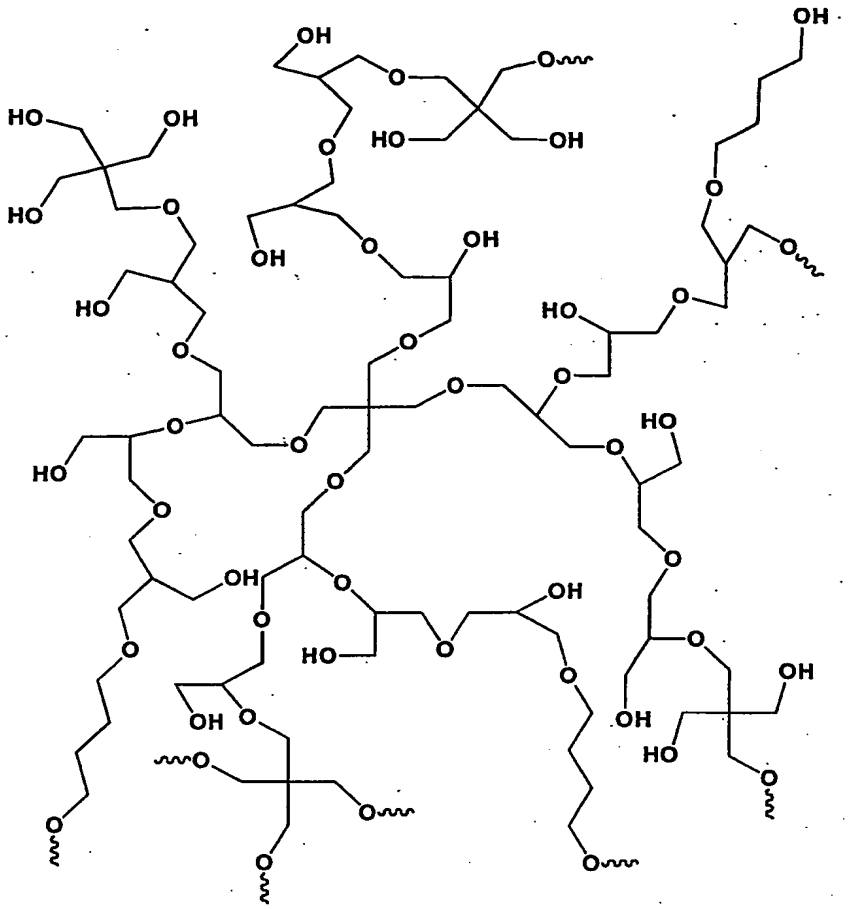
These first and second components can be used to generate a high molecular mass hyperbranched glycol polymer (HPEG) **D** terminated with hydroxyls in a single-step synthesis. This particular hyperbranched glycol is a semi-solid polymer with good solubility in water and various organic solvents. In still other embodiments, n (from **A** above) is greater than 1, for example, 20 or more, for example, 35, 50, 65, 80, or 100. In

other embodiments, a+b+c+d (from **B** above) is greater than 1, for example, 5, 15, 25, 50, 75, 100, 200, or more.

In particular, polyethylene glycol diglycidyl ether **A** with two epoxy groups and pentaerythritol ethoxylate **B** containing four hydroxyl groups are combined in THF and polymerized using boron trifluoride etherate for 3 hours at –10° C. Polymerization is continued for an additional 16 hours at room temperature. Upon first mixing, oligomeric cationic polymer **C** (oligo-HPEG) is generated, which grows in size over time. Upon quenching to stop polymerization, hyperbranched glycol polymer (HPEG) **D** is liberated.



C (oligo-HPEG)



D (HPEG)

A useful solvent for this reaction is THF. While it is possible to use methylene chloride as a solvent, doing so can yield hyperbranched glycols of lower molecular weight when compared to hyperbranched glycols made using THF as the solvent. Since it is believed that both secondary and tertiary oxonium ions participate in the polymerization, the process appears to combine the features of activated monomer (AM) mechanism (secondary oxonium ion) and the active chain end (ACE) mechanism (tertiary oxonium ion). These mechanisms are discussed in Biedron et al., *J. Polym. Sci. Pol. Chem.*, 1991, *29*, 619; Biedron et al., *Polym. Int.*, 1995, *36*, 73, and Bednarek, et al., *J. Polym. Sci. Pol. Chem.*, 1999, *37*, 3455. Both ^{13}C and ^{1}H NMR show the presence of both the $-OCH_2CH_2O-$ and $-OCH_2CH_2CH_2CH_2O-$ units. The presence of $-OCH_2CH_2CH_2CH_2O-$ confirms the incorporation of THF into the polymer. ^{13}C MR data showed that the $-OCH_2CH_2CH_2CH_2O-$ unit was absent from the polymer when

polymerization reaction was carried out in methylene chloride. The ratio of ethylene oxide backbone (69.8 ppm) unit to the tetrahydrofuran backbone (26.1 ppm) unit was 2.3 calculated using ^{13}C data.

The molecular mass of several hyperbranched glycols (namely, HPEG1 and HPEG2), determined using monodisperse, linear PEO as a standard in 0.1 M aqueous ammonium acetate solvent, are reported in TABLE 1 below. In addition, polydispersity, yield, hydrodynamic diameter are also reported. Additional details on the synthesis can be found in the Examples.

We have estimated the total number (core and shell) of hydroxyl groups as 104 OH groups/molecule for HPEG2, using the approach described in Sunder et al., *Macromolecules,* 2000, *33*, 7682.

^{13}C NMR data can be used to calculate the degree of branching (DB). We assigned the 72.2-72.7 ppm peak to the linear monomeric unit, whereas the peaks at 30.4 and 60.2-60.6 ppm were assigned to the termini of branches. The peaks at 29.4 and 72.4 ppm correspond to the dendritic core. Using peak integration with a 5 second delay between pulses, we calculated the degree of branching using the formula described by Hawker et al., *J. Am. Chem. Soc.* 1991, *113*, 4583, that $DB = (N_d + N_t) / (N_d + N_t + N_l)$, where N is the integrated value of the peak and d, l and t represent dendritic, linear and terminal unit, respectively.

We have found values for DB using the above described technique of about 0.68 and about 0.59 for HPEG1 and HPEG2, respectively. It has been shown by Radke et al., *Macromolecules* 1998, *31*, 239 the DBs approach 0.5 for hyperbranched polymers.

Dendrimers are substantially monodispersed, and typically have a polydispersity of less than about 1.02. Dendrimers are globular molecules, having a degree of branching that approaches 100%. In contrast, a linear polymer has a degree of branching of around 0%. Hyperbranched polymers, as described herein, are polymers having branches upon branches, and can be viewed as intermediate between traditional branched polymers and dendrimers. The degree of branching, which reflects the fraction of branching sites relative to a perfectly branching system (an ideal dendrimer), for a hyperbranched polymer, is greater than 0 and less than 1, with typical values ranging from about 0.4 to 0.6.

TABLE 1: Molecular Weight and Size Data for Several Hyperbranched Polymers

Entry	M_n (K)	M_w (K)	PDI (M_w/M_n)	yield (%)	HD[a] (nm)	CHD[b] (nm)	CHD4W[k] (nm)	Zeta Pl[m] (mV)
HPEG1[c]	38.1	72.1	1.89	92	30.9 ± 3.9	-	-	-
HPEG2[d]	24.7	41.3	1.67	90	24.7 ± 5.6	-	-	-
HPPEOX	67.3	120	1.78	44	48.7 ± 4.8	-	-	-
HPPMOX1	56.2	97.5	1.73	82	39.8 ± 3.9	-	-	-
HPPMOX2[e]	49.3	81.5	1.65	90	32.8 ± 6.3	-	-	-
HPPMOX3[f]	43.6	69.5	1.59	89	27.8 ± 5.7	-	-	-
BPLP4[g]	49.3	75.9	1.54	-	1328.8 ± 49.2	44.6 ± 3.4	43.7±4.1	-
BPLP3[h]	29.3	48.9	1.67	-	1033.3 ± 52.7	56.1 ± 6.7	61.3±6.2	4.69
BPLP2[i]	37.5	59.6	1.59	-	640.9 ± 39.4	56.2 ± 8.5	54.2±4.3	11.72
BPLP1[j]	42.6	69.4	1.63	-	607.6 ± 61.8	35.9 ± 5.3	36.9±3.7	-

a) HR =hydrodynamic diameter; measured at 50 μg/ml
b) CHD =hydrodynamic diameter of the complex: 25 μg DNA and 50 μg of polymer were mixed in 1ml 5% glucose solution
c) Prepared by the procedure outlined in Example 1, using 0.01 mol boron trifluoride etherate and a total reaction volume of 300 mL
d) Prepared by the procedure outlined in Example 1, using 0.02 mol boron trifluoro etherate and total reaction volume is 300 mL
e) Prepared by the procedure outlined in Example 8, using 10 ml 2-methyl 2-oxazoline and a polymerization time of 24 hours
f) Prepared by the procedure outlined in Example 8, using 7 ml 2-methyl 2-oxazoline and a polymerization time of 24 hours
g) BPLP4 is obtained as described in Example 5 by hydrolyzing HPPEOX (Example 4)
h) BPLP3 is obtained by hydrolyzing HPPMOX3
i) BPLP2 is obtained by hydrolyzing HPPMOX2
j) BPLP1 is obtained by hydrolyzing HPPMOX1
k) CHD4W = hydrodynamic diameter of the complex with DNA after 4 weeks
l) Size exclusion was performed in 0.1M ammonium acetate with poly(ethylene oxide) as molecular weight standards
m) Complex was made using 25 μg DNA and 50 μg of polymer were mixed in 1ml 5% glucose solution

In some embodiments, a hyperbranched glycol is made using glycerol diglycidyl ether and pentaerythritol ethoxylate in presence of boron trifluoro etherate as an initiator (HPGG). The resulting polymer is semi-solid and soluble in water, as well as various organic solvents. We have measured the total number of hydroxyl groups as 144 OH groups/molecule using the approach described earlier. The large number of hydroxyl groups can be explained by polymer branching. To calculate the degree of branching, we used ^{13}C NMR data. We have assigned the 72.1-72.8 ppm peak to the linear monomeric unit, whereas the peaks at 44-45 and 60.2 ppm were assigned to the termini of branches.

The peaks at 68 and 78 ppm correspond to the dendritic core. Using peak integration, we calculated the degree of branching using the formula DB = (Nd + Nt) / (Nd + Nt + Nl), where DB is the degree of branching, N is the integrated value of the peak, and d, l, and t represent the dendritic, linear and terminal units, respectively. The values obtained were 0.59 for HPGG1 and 0.49 for HPGG2. Properties are summarized in TABLE 2. Both polymers were synthesized under identical conditions using different amounts of the catalyst. The obtained DB values demonstrate the ability to control molecular architecture using various reaction conditions.

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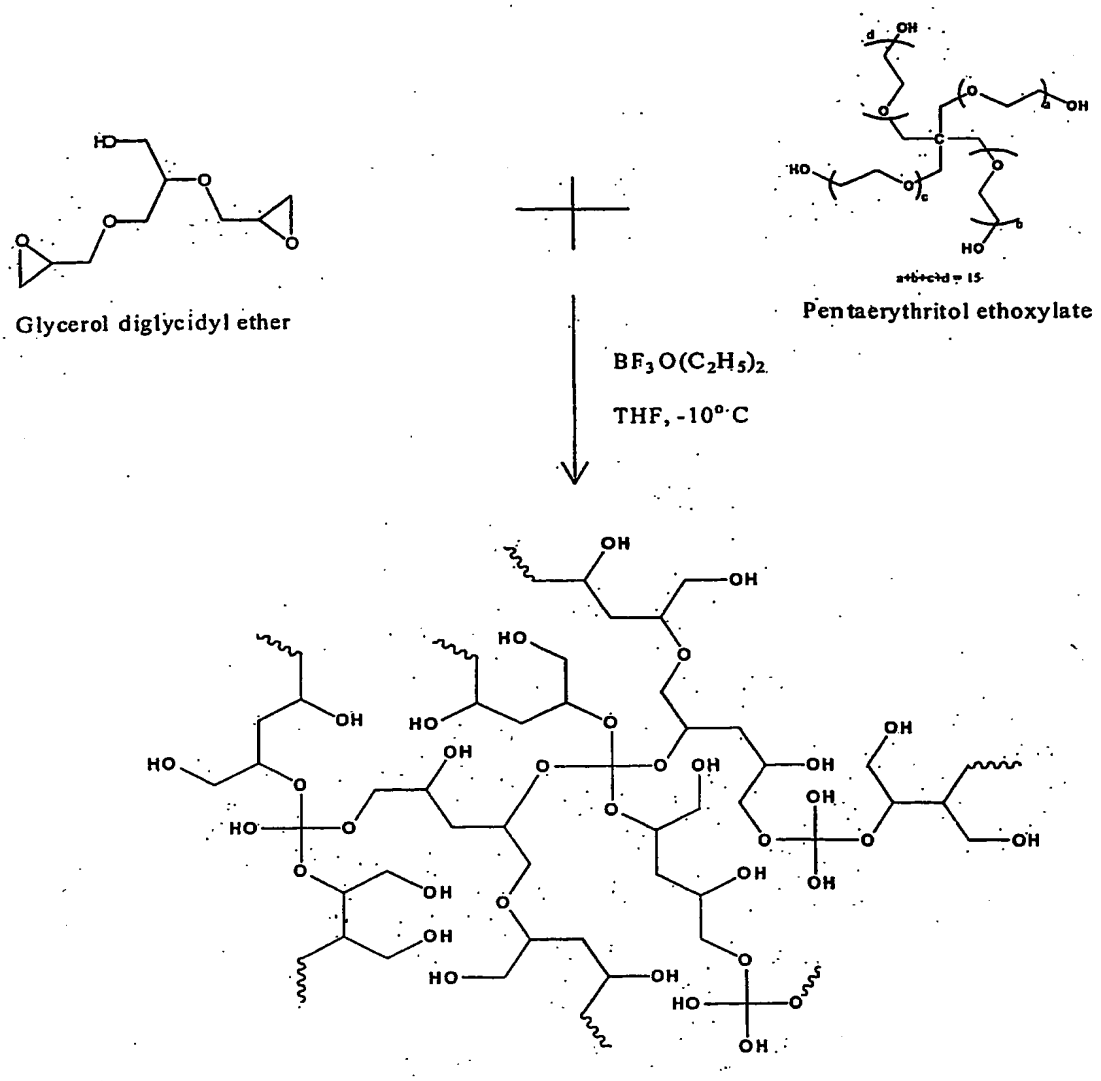


HPGG

Table 2. Characterization of Hyperbranched Polymers and Block Copolymers[a]

Entry	M_n (K)	M_w (K)	PDI (M_w/M_n)	yield (%)	HD[b] (nm)	CHD[c] (nm)
HPGG1[d]	34.3	48.4	1.99	90	30.9 ± 3.9	-
HPGG2[e]	29.8	41.3	2.20	93	24.7 ± 5.6	-
HPGMOX1[f]	46.2	89.2	1.93	82	39.8 ± 3.9	-
HPGMOX2[g]	40.7	64.2	1.85	90	32.8 ± 6.3	-
HGLP6[h]	39.4	68.6	1.74	-	1447.2 ± 64.2	47.6 ± 5.2
HGLP7[i]	34.8	43.9	1.77	-	1114.8 ± 59.7	65.2 ± 4.7
BPEI	9.5	23.4	2.46	-	197.5 ± 4.7	120.2 ± 10.6
HPGBPEI[j]	40.2	82.3	2.04	-	177.5 ± 2.3	56.2 ± 4.3

(a) Size exclusion was performed in 0.1M ammonium acetate with poly(ethylene oxide) as molecular weight standards

(b) HD =hydrodynamic diameter; measured at 50 μg/ml

(c) CHD =hydrodynamic diameter of the complex: 25 μg DNA and 50 μg of polymer were mixed in 1ml 5% glucose solution

(d) Prepared by the procedure outlined in Example 10, using 0.01 mol boron trifluoride etherate

(e) Prepared by the procedure outlined in Example 10, using 0.02 mol boron trifluoro etherate

(f) Prepared by the procedure outlined in Example 12, using 10 ml 2-methyl 2-oxazoline and a polymerization time of 24 hours

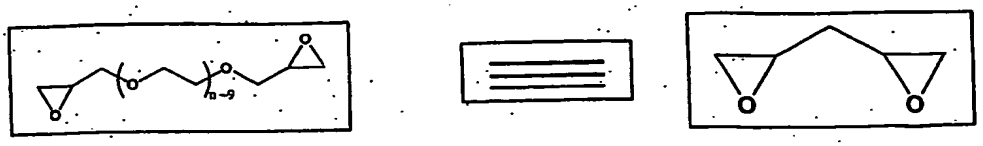
(g) Prepared by the procedure outlined in Example 12, using 7 ml 2-methyl 2-oxazoline and a polymerization time of 24 hours

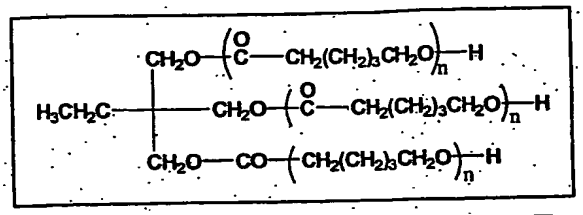
(h) HGLP6 is obtained by hydrolyzing HPGMOX1 (Example 13)

(i) HGLP7 is obtained by hydrolyzing HPGMOX1 (Example 13)

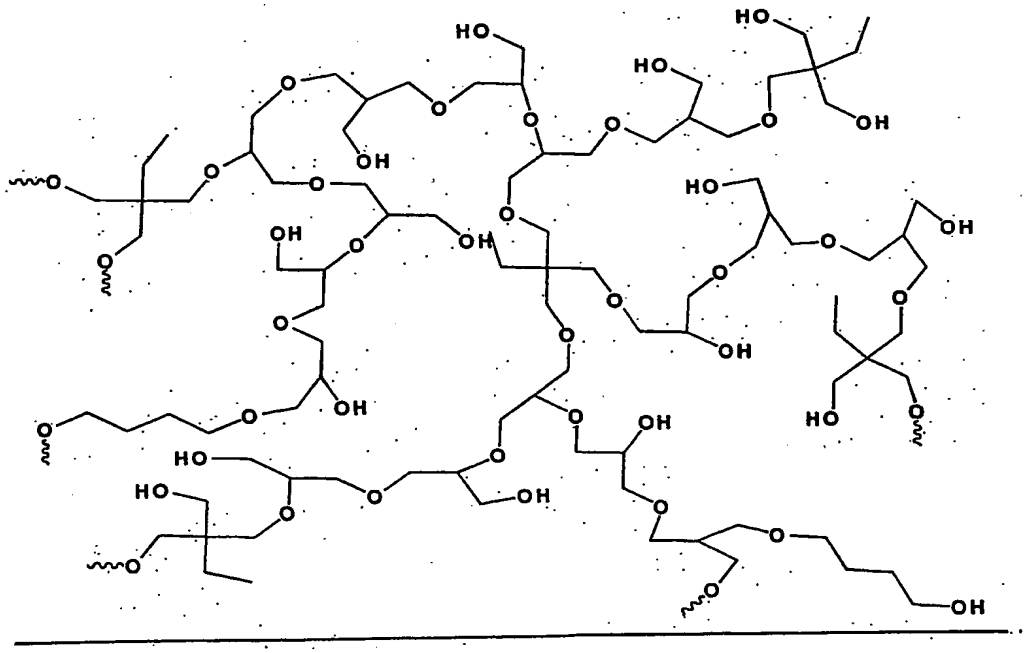
(j) HPGBPEI is prepared by the procedure outlined in Example 17

In another embodiment polyethyleneglycol diglycidyl ether and polycaprolactone triol are polymerized (shown below). The resulting polymer (HPCG) swells in water, but is not soluble, which is consistent with the incorporation of both a hydrophobic caprolactone moiety and hydrophilic glycol moiety. This synthesis procedure shows the possibility of making hyperbranched, surface-functionalized biodegradable polymers in one step.



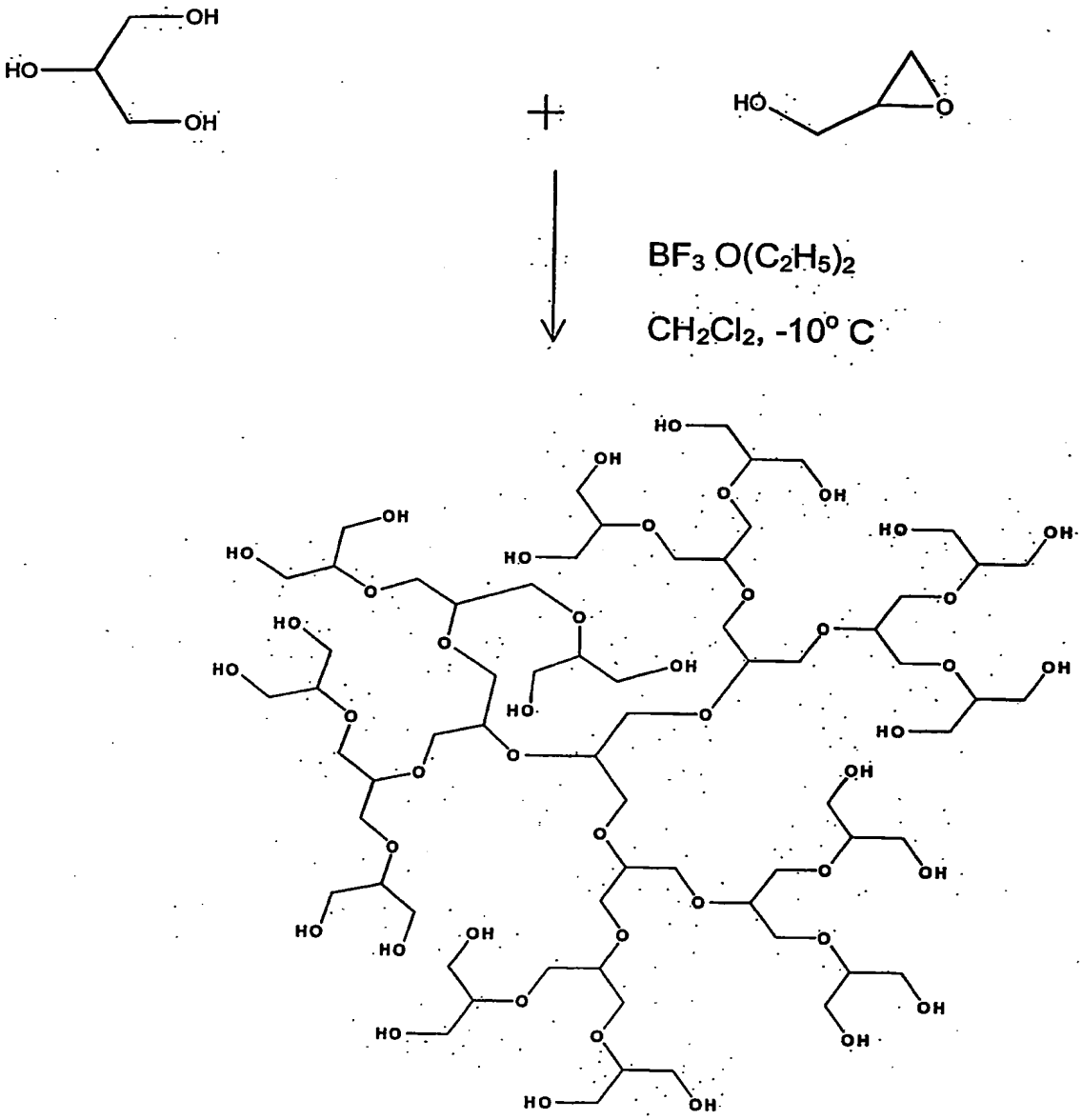


Polycaprolactone triol



HPCG

In another embodiment, a monomer with one epoxy group, e.g., glycidol, is reacted with the multiple hydroxyl group monomer glycerol in methylene chloride. The polymer produced (HPG) (shown below) is water soluble and has a low molecular weight (< 5K). Other low molecular weight materials having hydroxyl groups in the outer shell can be prepared from glycerol and the other diepoxy monomers described above.



HPG

Functionalization of Hyperbranched Polyglycols

Referring to Figs. 2-4 and again to Fig. 1, hyperbranched glycols (Fig. 1) can be made more reactive by reacting at least some hydroxyl groups of the hyperbranched glycol polymer with an activating component that enhances the reactivity of the hyperbranched glycol polymer towards substitution and/or elimination reactions. For example, the hyperbranched glycol polymer (Fig. 1) can be activated by reacting the

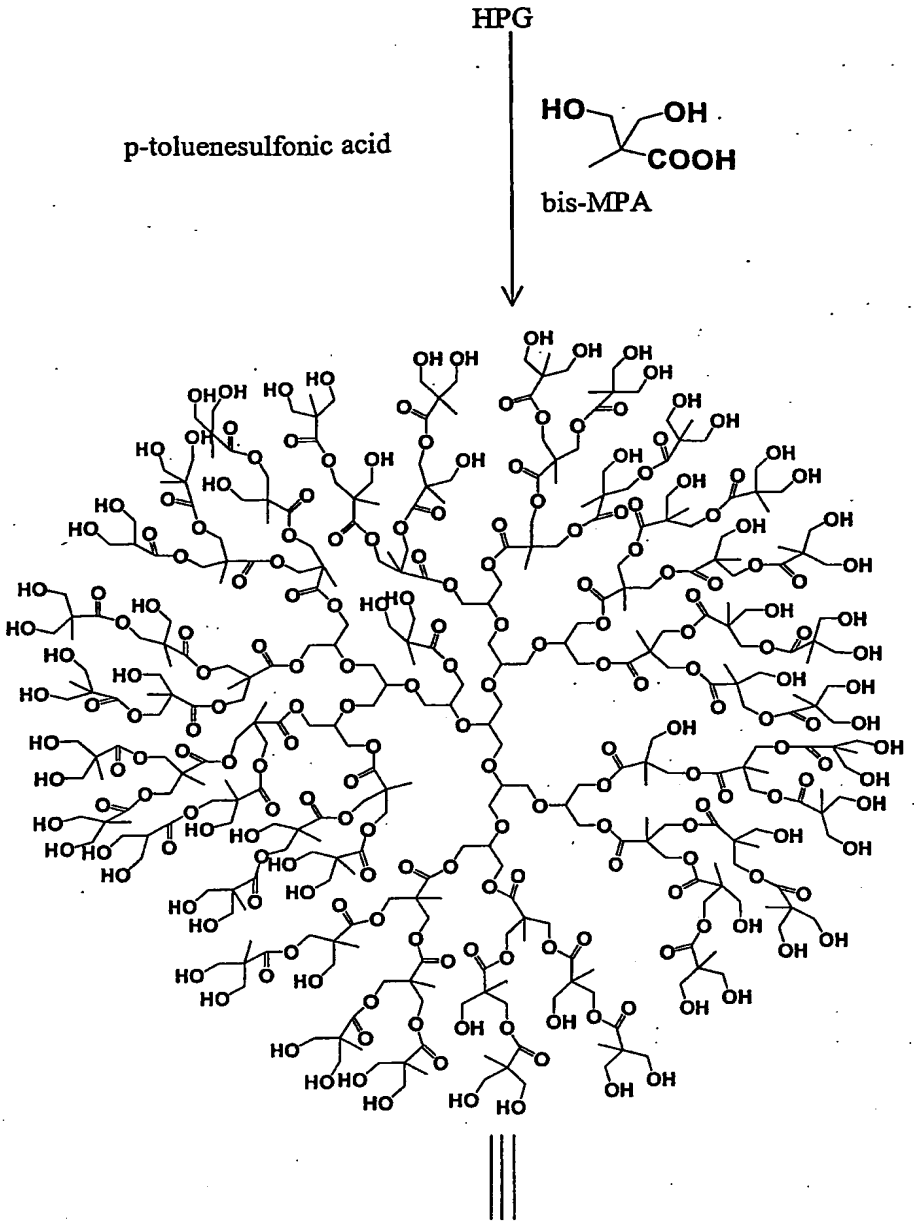
glycol polymer with a proton source (as shown in Fig. 2), reacting the glycol polymer with a base and deprotonating (as shown in Fig. 3) at least some of the hydroxyl groups of the glycol polymer, or by reacting the glycol polymer with an activating moiety ϕ (Fig. 4) that becomes grafted to the glycol polymer. While Figs. 2-4 show only an outer shell that is activated, it will be understood by those skilled in the art that it is possible to also activate those hydroxyl groups in the core as well. Suitable acids include strong organic acids, for example, perfluoroacetic acid and/or mineral acids, for example, sulfuric acid. Suitable bases include hindered alkoxides, for example, potassium t-butoxide, calcium hydride, and lithium aluminum hydride. In addition, the alkoxide can be produced by treatment with an active metal, for example, sodium or lithium metal.

The activating component enhances the reactivity of the hyperbranched glycol polymer towards nucleophilic substitution, electrophilic substitution, and/or elimination reactions. For example, the protonated hydroxyl groups of Fig. 2 are good leaving groups for an attacking nucleophile, for example, an alcohol, to form an ether. Likewise, the protonated hydroxyl groups of Fig. 2 are good leaving groups for an attacking nucleophile, for example, a water molecule, to form an olefin that can, for example, undergo other reactions, for example, cross-linking or additions reactions. The alkoxide groups of Fig. 3 are good nucleophiles that can be alkylated, for example, with an organic halide.

Suitable activating moieties ϕ that become grafted to the glycol polymer include components with latent functionality that can undergo further substitution or elimination reactions such as grafting reactions. Such moieties include, for example, 4-chloromethyl benzoyl chloride, p-toluene sulfonic acid, and tin(II) hexanoate.

Polymers can be grafted to any of the structures shown in Figs. 1-4. Suitable polymers are degradable or cleavable polymers and non-degradable polymers. Any of the structures shown in Figs. 1-4 can also initiate polymerization of monomeric species, thereby grafting polymers onto at least a portion of their architecture.

In some embodiments, the hyperbranched glycol polymer is esterified with a small or large molecule, such as derivatives of benzoyl chloride, sulfonyl chloride, acetyl chloride, vinyl sulfonyl chloride, chloroformate, and polyethylene glycol chloroformate. In other embodiments, the glycol polymer is reacted with a small or large isocyante, forming a polyurethane. In a particular embodiment, HPG was esterified with bis(hydroxymethyl) propionic acid (bis-MPA) in the presence of p-toluenesulfonic acid to get a high molecular weight hyperbranched polyester (HPET).



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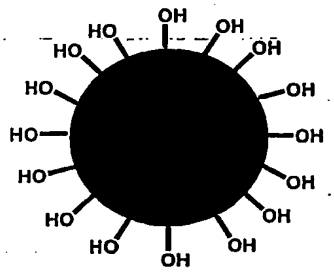
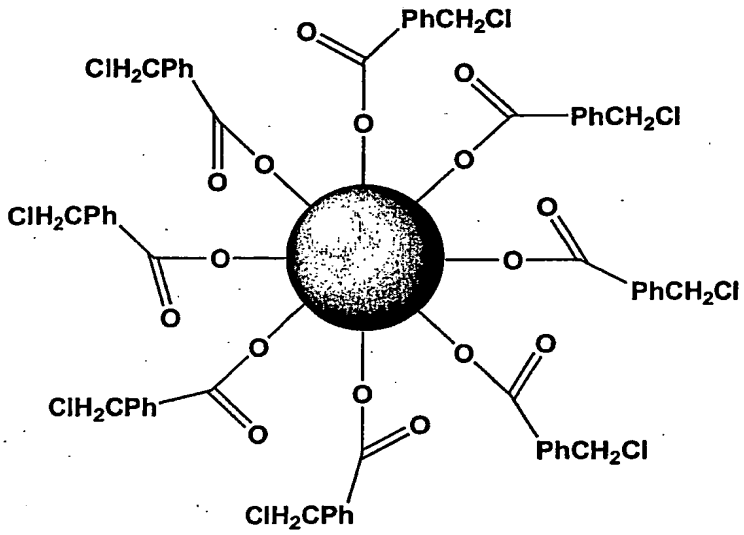


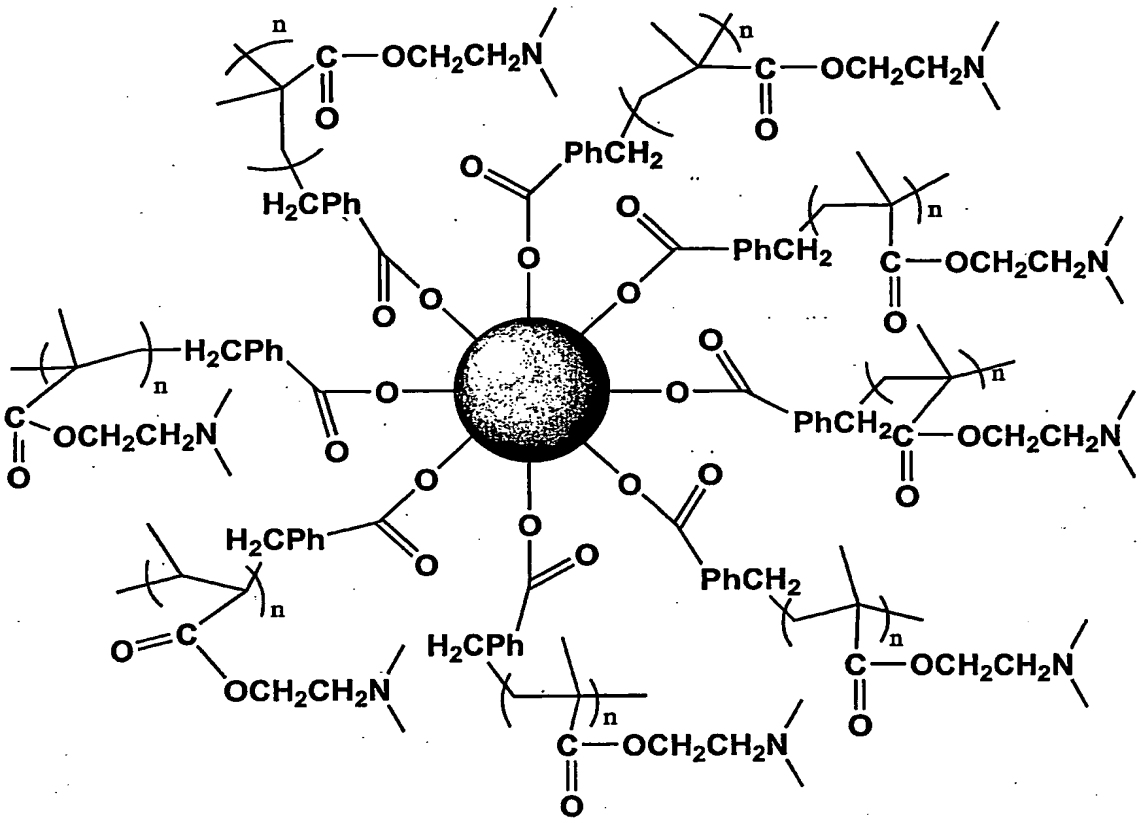
HPET

In some embodiments, block copolymers of the hyperbranched glycol polymers (HPEGs) described above and, for example, polyethyleneimines (HPEG-PEI), are prepared by first activating hydroxyl groups of the hyperbranched glycol polymer with either 4-chloromethyl benzoyl chloride or p-toluene sulfonyl chloride. Chloromethyl benzene and toluene sulfonic acid derivatives are known to efficiently catalyze the ring opening polymerization of 2-oxazolines and that the chain end of the growing poly (oxazoline) can be functionalized by termination or by initiation. See, for example, Kobayashi, *Prog. Polym. Sci.* 1990, *15*, 751 and Akiyama, et al., *Macromolecules,* 2000, *33*, 5841.

Reacting hyperbranched glycols (HPEGs, Fig. 1) described above with 4-chloromethyl benzoyl chloride, produces a chloromethylbenzyl grafted macroinitiator (HPEG-Cl) **E**. Macroinitiator **E** can then be polymerized with any vinyl compound, for example, dimethylamino ethyl methacrylate (DMEMA) using copper bromide and pentamethylene-diethylene triamine (PMDETA) in THF at 60° C, yielding polydimethylamino ethyl methacrylate grafted polymer (HPEG-DMEMA) **F**. Polymer **F** was characterized by ^{1}H NMR, which showed that more than about 90% of chloromethylbenzyl groups react to initiate polymerization. Polymer **F** can be quaternized with, for example, methyl iodide via dimethylamino groups to make a cationic corona polymer (not shown).



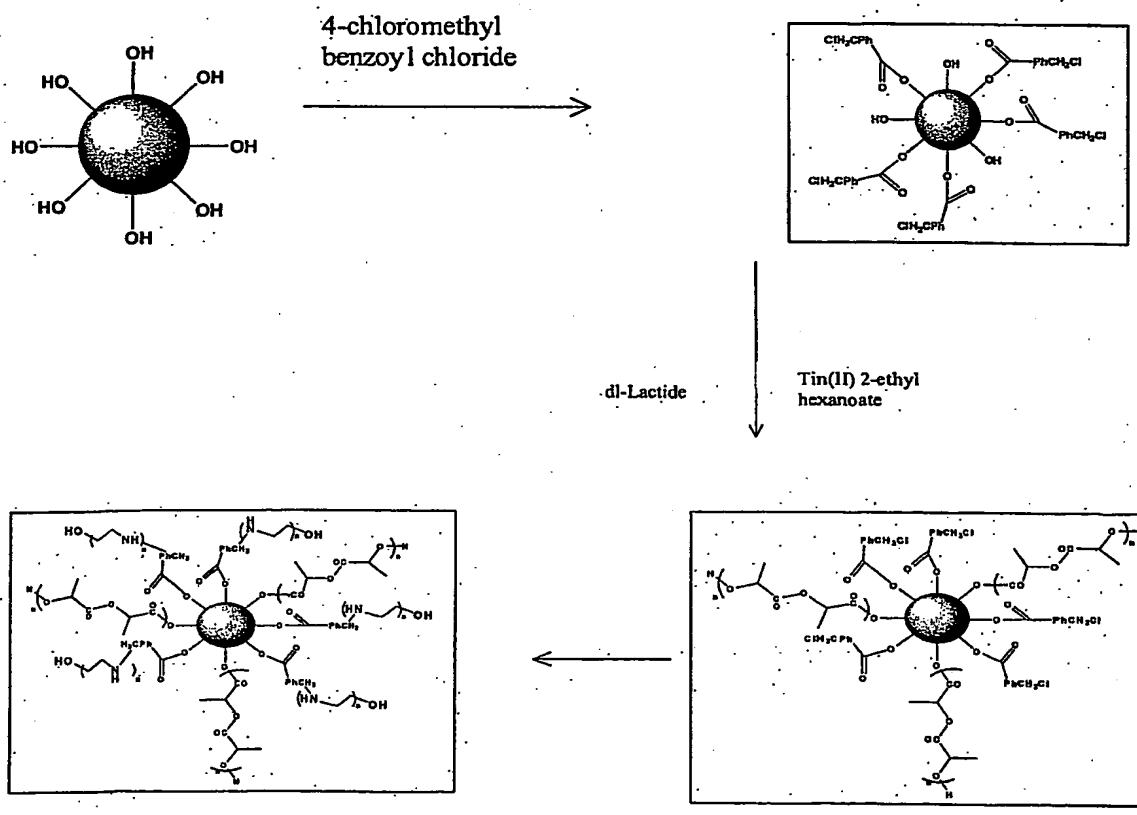
E (HPEG-Cl)



F (HPEG-DMEMA)

Chloromethylbenzyl grafted macroinitiator **E** was characterized by ^{13}C and ^{1}H NMR. We established by ^{13}C NMR data that approximately 75% of the hydroxyl groups are converted to chloromethylbenzyl derivatives. This suggests that not all hydroxyl groups of the hyperbranched glycol have the same reactivity. It seems likely that the surface hydroxyl groups are more reactive than those disposed in the polymer core. This interpretation is in agreement with a study that suggested that the reactivity of the chain end is lower in the case of hyperbranched polymers than dendrimers (see, for example, Frechet, et al., *J. Macromol. Sci.-Pure Appl. Chem.*, 1994, *A31*, 1627). We have discovered, however, that under some conditions, essentially all the hydroxyl groups can be functionalized. For example, reacting a large excess of p-toluene sulfonyl chloride with a hyperbranched glycol polymer resulted in a nearly 100% conversion of all of the hydroxyls groups.

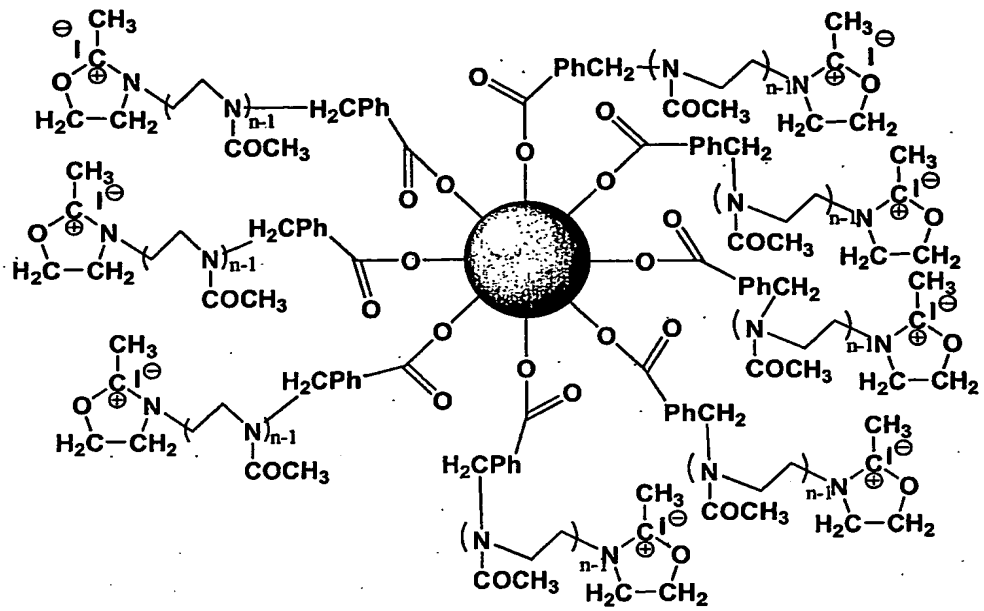
In particular embodiments, HPEG was reacted with two molar excess of 4-chloromethyl benzoyl chloride. NMR data suggests that only 75% hydroxyl groups were converted to chloromethyl benzoyl derivatives. The resulting derivative was then reacted with lactide monomer using tin(II) ethyl hexanoate as catalyst (shown below). The unreacted hydroxyl groups of HPEG-Cl, in presence of tin hexanoate, react with lactide, thereby grafting biodegradable polylactide chains onto the hyperbranched polymer. This result indicates the availability of reactive hydroxyl groups in HPEG-Cl. To test the reactivity of chloromethyl benzene moieties, the chloromethylated HPEG-b-polylactide was further reacted with 2-methyl oxazoline in presence of KI to form the poly(2-methyl oxazoline) block shown below. This result indicates the diversity of hyperbranched polymers that can be prepared. Such hyperbranched polymers are useful in various biomedical applications.



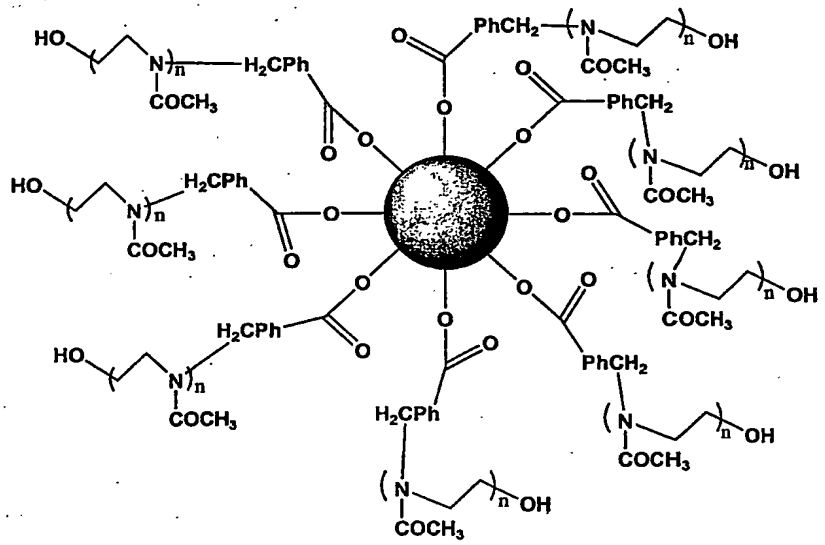
In other particular embodiments, a HPEG-tosylate polymer (not shown) formed by the reaction of a hyperbranched glycol polymer (HPEG) and p-toluene sulfonyl chloride is polymerized with 2-ethyl-2-oxazoline in acetonitrile at reflux. The hyperbranched glycol polymer grafted with poly(2-ethyl-2-oxazoline), HPPEOX, can be precipitated in ethyl ether, dried in-vacuum and characterized. The molecular weight, polydispersity, yield, and hydrodynamic diameter for HPPEOX are reported in TABLE 1. The polymer can be hydrolyzed with sodium hydroxide in a ethanol/ethylene glycol solvent mixture, resulting in a HPEG-block-linear polyethyleneimine polymer (BPLP), using a procedure described by Akiyama, et al., *Macromolecules,* 2000, *33*, 5841. Purification of the BPLP is accomplished by ultrafiltration followed by lyophilization. ^{1}H NMR shows complete hydrolysis.

In another embodiment, **E** (HPEG-Cl) is polymerized with 2-methyl-2-oxazoline in benzonitrile using potassium iodide. It is believed that the polymerization of 2-methyl-2-oxazoline using **E**, proceeds via an ionic or covalent mechanism, depending upon the counter ion of the polymer chain end. For additional details on proposed mechanisms, see, for example, Kobayashi et al., *Macromolecules,* 1992, *25*, 3232; Saegusa et al., *Macromolecules,* 1972, *5*, 359; Saegusa et al., *Macromolecules,* 1973, *6*, 315; and Saegusa, et al. *Makromolekulare Chemie-Macromolecular Chemistry and Physics,* 1976, *177*, 2271.

It has been reported that grafting reactions based on chloromethylbenzyl derivatives can result in low monomer conversion and that the reaction proceeds via covalent propagation. The reactivity of the initiator, however, usually increases by exchanging the chloride anion with a better leaving group. For additional details on this presumption, see, for example, Saegusa et al., *Macromolecules,* 1973, *6*, 315 and Saegusa et al., *Makromolekulare Chemie-Macromolecular Chemistry and Physics,* 1976, *177*, 2271. Potassium iodide, for example, is used to exchange chloride anion with iodide, which usually improves conversion and polymerization yields. We prepared block copolymers of **E** and 2-methyl-2-oxazaline under several different conditions, yielding hyperbranched glycol polymers grafted with poly(2-methyl-2-oxazoline), HPPMOX, having a general structure **H**. To liberate **H**, living polymer **G** is quenched with water. The molecular weight, polydispersity, yield, and hydrodynamic diameter for several HPPMOX polymers (HPPMOX 1-3) are reported in TABLE 1.



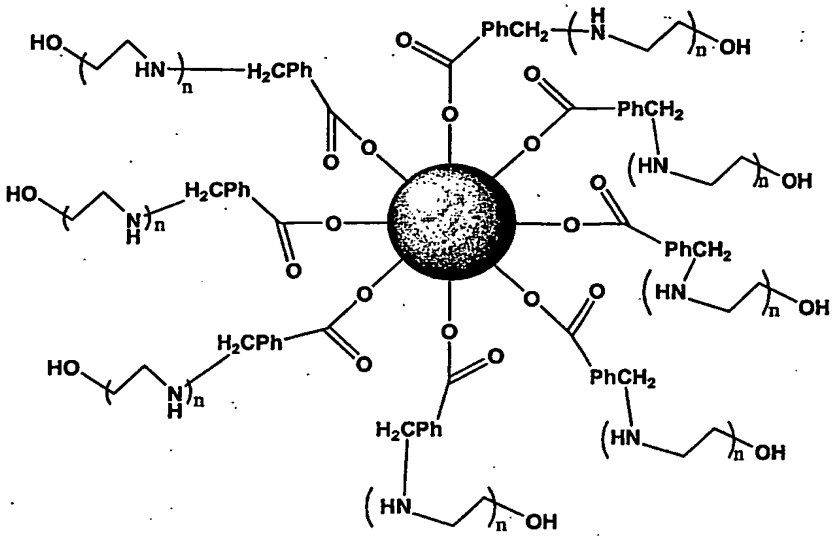
G (HPPMOX before quenching)



H (HPPMOX)

Polymer **H** was isolated by ethyl ether precipitation. Hydrolysis of polymer **H**, yields a HPEG-block-polyethyleneimine polymer (BPLP). BPLP polymers have the general structure **I** shown below. The molecular weight, polydispersity, yield and hydrodynamic diameter for several BPLP polymers (BPLP 1-3 derived from polymer **H** and BPLP4 derived from the grafted ethyl oxazaline polymer, HPPEOX, discussed above,

but not shown) are reported in TABLE 1. Analysis using NMR before (H) and after (I) NaOH hydrolysis showed that methyl protons of the acetyl group appearing at 1.9 ppm were undetectable after the hydrolysis and methylene proton adjacent to nitrogen are shifted from 3.4 to 2.7 ppm, suggesting nearly complete hydrolysis.

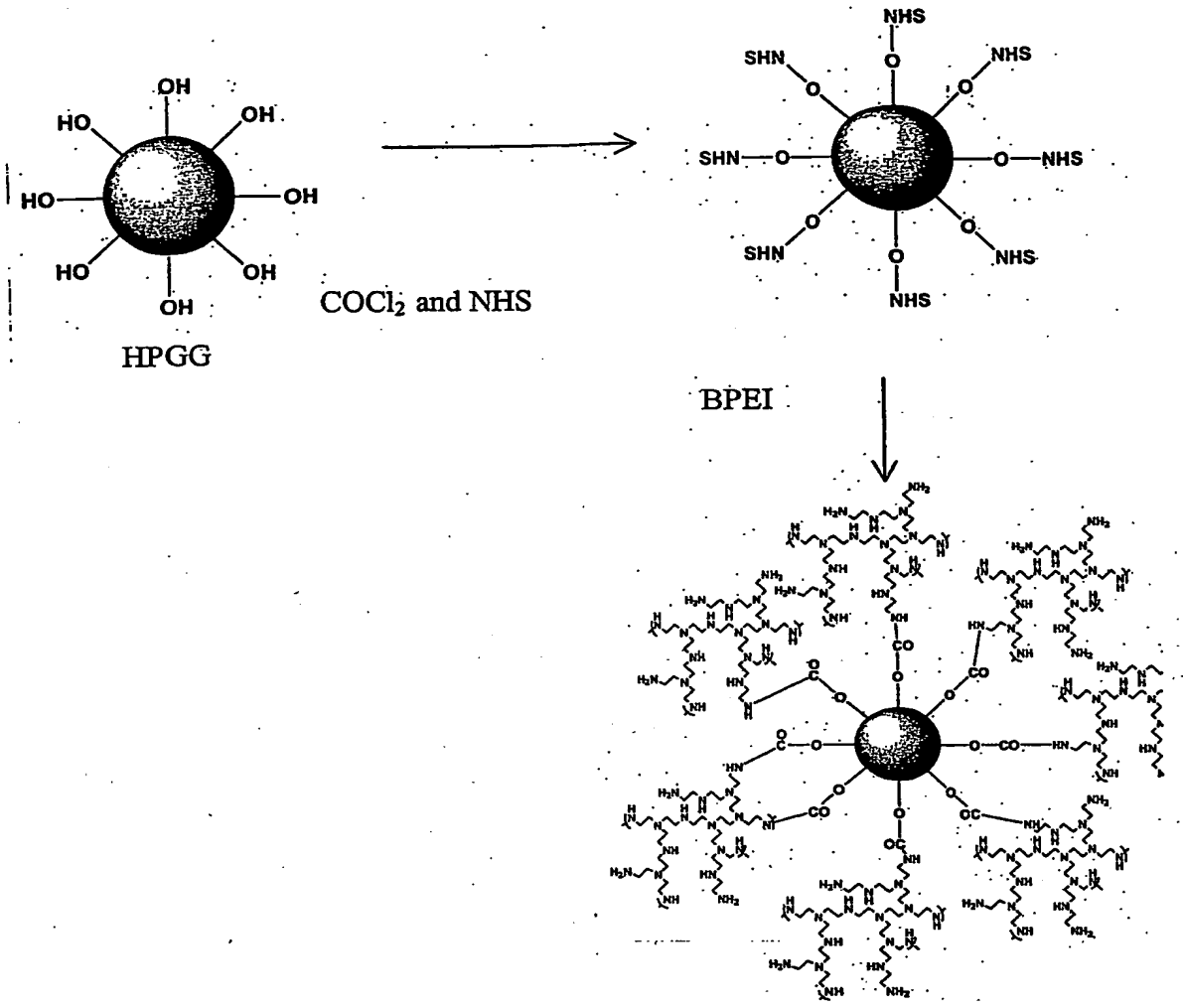


I (BPLP)

In certain embodiments, HPGG-Cl was polymerized with 2-methyl oxazoline in benzonitrile in the presence of potassium iodide, yielding block copolymer HPGGMOX. This block copolymer was then hydrolyzed with sodium hydroxide in ethanol/ethylene glycol mixture, resulting in block copolymer HPGG and LPEI (BPLP). Additional details are in TABLE 2.

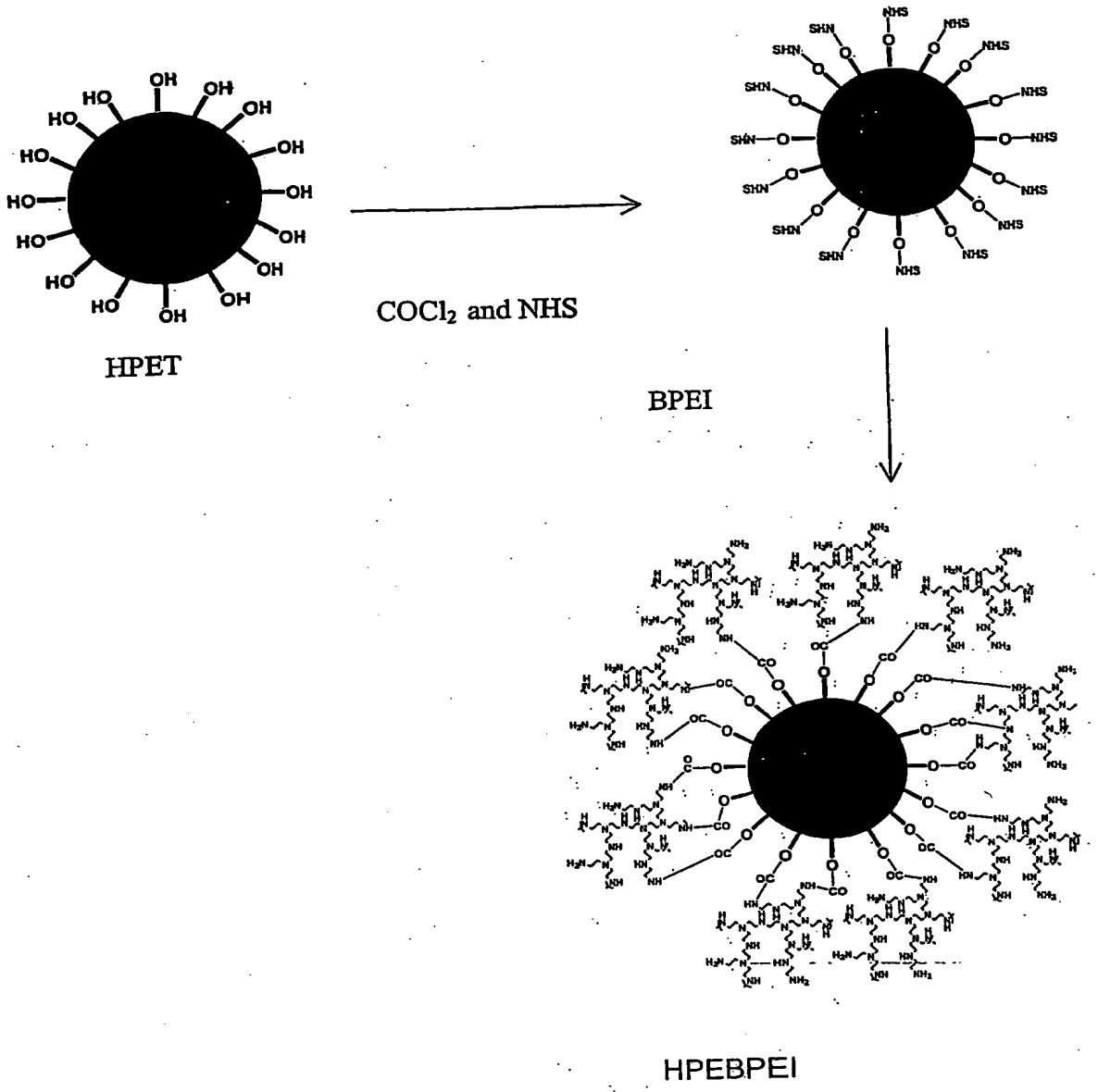
In some embodiments, chiral oxazolines are utilized, yielding chiral hyperbranched polymers that are useful in applications such as delivering therapeutic agents to the body of a mammal or packing for an HPLC column to facilitate the chiral separations. Suitable chiral oxazalines include 2-[1-(Hydroxymethyl)ethyl]-oxazoline.

In particular embodiments, block copolymers are made using hyperbranched glycol polymers and commercially available branched polyethyleneimine (BPEI). For example, to make a HPGG block copolymer with branched polyethyleneimine (BPEI), we first made the N-hydroxy succinimide (NHS) derivative of HPGG (shown below). The NHS-HPGG is then reacted with BPEI, forming HPGBPEI.



HPGBPEI

In certain embodiments, block copolymers are made using a hyperbranched polyester polymer and a commercially available branched polyethyleneimine (BPEI). To make a HPET block copolymer (shown below) with branched polyethyleneimine (BPEI), the N-hydroxy succinimide derivative of HPET is first prepared. The NHS-HPET is then reacted with BPEI to form HPEBPEI.



Adducts of Hyperbranched Polymers and Nucleic Acids

To test the DNA condensing properties of some of the above described hyperbranched polymers, we measured the hydrodynamic diameters of the BPLP polymers and compared these with the hydrodynamic radii of resulting complexes of those polymers with negatively charged DNA. The results are summarized in TABLE 1 and TABLE 2.

We obtained complexes between 25 μg of plasmid DNA (pCMV-Luc, 5.12 kb) and 50 μg of the BPLP polymers in non-ionic, isotonic solution (5% glucose in water).

Upon complex formation with DNA, the diameter of the complex decreased sharply. All BPLP complexes appear to behave like cationic polymers. As nucleic acids are negatively charged, positively charged BPLP can form complexes with nucleic acids by electrostatic interaction. The inner hyperbranched core is polyglycol based, and is neutral. The cationic part is the polyethyleneimine formed after hydrolysis of the block copolymer formed between a hyperbranched glycol polymer and poly(methyl oxazoline). It appears that the hyperbranched glycol inner core does not interfere with complex formation, but rather helps to stabilize the particles in solution for at least 30 days. The measured zeta potentials (TABLE 1) of complexes appear to suggest that the zeta potential can be controlled by changing the density of cationic residues. We have established that a higher molecular weight polymer yields a more positive zeta potential than lower molecular weight polymer.

Cytotoxicity of Hyperbranched Polymers

Cytotoxicity was measured for the BPLP (see TABLE 1) polymers and commercially available LPEI to test whether toxicity can be reduced in the presence of a hyperbranched core. We measured the toxicity using COS-1 cells in the presence of serum at 48 hours after adding the polymers to the cells. The cytotoxicity was measured using CytoTox96® non-radioactive assay (Promega) and the results are summarized in Fig. 5, which shows that BPLP polymers (TABLE 1) had a very low toxicity even at a high concentration of 0.5 mg/ml. It is known that PEG grafted BPEI (with PEG in the outer shell) is less toxic than BPEI alone (see, for example, Petersen et al., *Macromolecules,* 2002, *35*, 6867). In our present case, the hyperbranched glycol is in the inner core, and yet it decreases toxicity in a similar manner. Commercially available linear PEI (LPEI) (MW 22,000), was shown to have a much higher cytotoxicity than any of the BPLP polymers used in this study.

These results suggest that the presence of a hyperbranched glycol core does indeed reduce cytotoxicity. At the same time, particle size and stability data suggest that BPLP polymers behave differently than PEG-grafted BPEI. The hydrodynamic diameters of BPLP polymer complexes with DNA were much smaller in size than those formed between PEI-g-PEG and studied in, for example, Petersen et al. *Bioconjugate Chem.,* 2002, *13*, 845. In addition, these complexes were stable for 30 days without a significant change in hydrodynamic diameter. This suggests that the presence of polyethylene glycol chains reduces cytotoxicity in a manner similar to the conventional PEI-g-PEG, and even

more efficiently when compared to linear polyethyleneimine, as described by Jeong et al., *J. Control. Release*, 2001, *73*, 391. In addition, these results suggest that the complex condensation property of either BPLP is greater than PEI-g-PEG, as described by Kichler et al., *J. Control. Release*, 2002, *81*, 379.

Transfection

Fig. 6, is a bar graph that illustrates the transfection efficiency of pCMV-Luc vector into COS1 cells for commercial LPEI (MW 22,000) as compared with two BPLP polymers, BPLP3 and BPLP2. At higher concentrations, the BPLP polymers show higher transfection efficiency than LPEI. Presumably due to the presence of a hyperbranched glycol core, a higher amount of block copolymer was required for higher transfection efficiency. The amount of polymer used for condensation of DNA showed lower cytotoxicity than the same amount of LPEI, as is evident from the data that are presented in Fig. 5. Additional details can be found in Example 23. The lower transfection efficiency of the pegylated PEI appears to be due to masking of PEI charge, resulting in the inhibition of the non-specific ionic interaction between the polycation-DNA complex and the cell surface.

Uses in RNAi

Small interfering RNA (siRNA) expressing plasmid DNA can be constructed using standard technique, such as by using pSuper-retro-GFP (Oligoengine, Seattle, WA). For example, nucleotide targeted siRNA sequences can be designed against a target sequence in the pGL3 basic vector firefly luciferase gene with loop sequence TTCAAGAGA. Any of the polymers described herein can be used to deliver siRNA-encoding plasmid DNA. Generally, complexes between hyperbranched polymers are made by mixing the plasmid DNA and with the polymer, and incubating with cells. After incubating, the total number of cells in each well are counted and the cells are lysed using luciferase assay lysis buffer (Promega).

Applications

Fig. 7 schematically illustrates some possible applications for the hyperbranched polymers described herein. Since the disclosed hyperbranched polymers include a highly adaptable, readily functionalizable architecture system that may be tailored, many useful applications are possible. For example, the hyperbranched polymers can be covalently

bound to or may form an adduct with a moiety for the purpose of delivering that moiety to the body of a mammal. For example, an MRI contrast agent 20, an optical imaging agent 22, a targeting moiety 24, a cleavable peptide or a therapeutic agent 30, for example, a nucleic acid, can be delivered to the body of a mammal using the new hyperbranched polymers described herein as delivery agents. These applications are, in part, made possible by the fact that the size of the polymer is readily modified, and the hydrophilicity of the polymer is readily adaptable, for example, by modification of the outer shell. This is coupled with a relatively low toxicity.

The new hyperbranched polymers include an inner core and an outer shell. The outer shell includes, for example, hydroxyl groups bound to an outer portion of the core. The hyperbranched polymers can be non-degradable or they may be biodegradable. For example, a portion of the hyperbranched polymer, for example, an outer shell, can be degradable or cleavable. For example, the outer shell can be made biodegradable by grafting onto any of the hyperbranched polymers described herein a degradable polymer. Examples of biodegradable polymers include polycaprolactone, polylactic acid, glycolic acid, and copolymers of lactic acid and glycolic acid or caprolactone.

EXAMPLES

The invention is further described in the following examples, which do not limit the scope of the invention described in the claims.

The examples given below describe the production, on a laboratory scale, and the characterization of hyperbranched glycols and functionalized glycols, including polymer-grafted hyperbranched glycols. Certain reactions, cytotoxicity, transfection efficiency, and utilization data of some of the new polymers are also presented.

The following materials were used in the examples. Anhydrous tetrahydrofuran, anhydrous methylene chloride, 2-methyl 2-oxazoline (98%), 2-ethyl 2-oxazoline (99+%), potassium iodide, glycerol (99.5%), glycidol (96%), benzonitrile (99%), polyethylene glycol diglycidyl ether (MW 526), pentaerithrytol ethoxylate (MW 797), boron trifluoride etherate, 4-chloromethyl benzoyl chloride, Copper bromide, pentamethyldiethylene triamine (PMDETA), 2-(Dimethylamino) ethyl methacrylate (DMAEMA) were purchased for Aldrich (Milwaukee, WI. Petroleum ether, diethyl ether was reagent grade and purchased from Fischer Scientific. All the reagents were used as received.

The molecular mass of polymers was estimated using a PL AQUAGEL-OH® 40 8 μm column (Polymer Laboratories Inc, Amherst, MA) eluted with 100 mM ammonium acetate (pH = 7) on a Varian ProStar® HPLC coupled with RI detector at 30 °C. Molecular mass was measured using polyethylene oxide standards. Weight average molecular mass was computed using a standard approach. Proton and ^{13}C NMR were recorded using D_2O solutions by Spectral Data Service (Champaign, IL) at 400 MHz for ^{1}H and 100 MHz for ^{13}C spectra, respectively.

COS-1 cells were propagated in Dulbecco's modified Eagle's medium with 4 mM L-glutamine, 1% penicillin-streptomycin and 10% fetal bovine serum. Cells were incubated at 37°C in 5% CO_2. For viability study, cells were maintained in DMEM with 4 mM L-glutamine, 1% penicillin-streptomycin and 10% fetal bovine serum.

The reporter gene plasmid, pCMV-Luc (3.2 MDa, 5.12 kb) encoding firefly luciferase was propagated in *E. coli*, DH5α and purified by column separation (Megaprep, Qiagen, Valencia, CA). Plasmid integrity was confirmed by gel electrophoresis in agarose. DNA concentration and purity was determined by measuring absorbance at 260 nm and 280 nm.

Example 1: Synthesis of HPEG in THF

In a dry two neck round bottom flask fitted with dropping funnel 17 g (0.021 mol) pentaerithrytol ethoxylate and 10 g (0.019 mol) polyethylene glycol diglycidyl ether was added in argon atmosphere. To this monomer mixture 300 ml of anhydrous tetrahydrofuran (THF) was added by cannula. The monomer solution was kept at –10 °C for 30 minutes. To the cold monomer solution boron trifluoride solution in 10 ml anhydrous THF was added drop wise over a period of 1 hour. Two different catalyst concentration 0.02 and 0.01 mol BF_3 was used to get two different molecular weight polymers. The polymerization was continued for 3 hours at –10 °C and additional 16 hours at room temperature. The polymerization was quenched using a trace amount of acidic water. The polymer was purified by repeated precipitation from petroleum ether and dried at vacuum. The dried polymer is nearly colorless viscous material. The polymers readily dissolve in water and various organic solvents. Yield was 25 gm. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.62 (-$OCH_2\underline{CH_2}\underline{CH_2}CH_2O$-), δ 3.4-3.42 (-$OC\underline{H_2}$- in THF-THF homodiad), δ 3.43-3.47 (-$OC\underline{H_2}$- in THF-EO heterodiad), δ 3.63 (-$OC\underline{H_2}$- in EO-THF heterodiad), δ 3.67 (-$OC\underline{H_2}$- in EO-EO homodiad), δ 3.75 (HO-$C\underline{H_2}$- and

–CHO(CH2OH)). ^{13}C NMR (100 MHz, DMSO-d_6) : δ 26.16 (-OCH$_2$CH$_2$CH$_2$CH$_2$O-), δ 29.3 (C (CH$_2$O-)$_4$), δ 30.4 (-C (CH2OH)$_3$), δ 60.29 (-CH$_2$CH$_2$OH), δ 60.62 (-CHCH$_2$OH), δ 68.61 (C (CH$_2$O-)$_4$), δ 69.62 (-OCH$_2$CH$_2$- in EO-THF heterodiad), δ 69.53 (-OCH$_2$CH$_2$- in THF-THF homodiad), δ 69.86 (-OCH$_2$CH$_2$- in EO-EO homodiad), δ 70.18 (-OCH$_2$CH$_2$- in THF-EO heterodiad), δ 70.58 ((-CH$_2$CH$_2$OH), δ 72.25 (-CHOH), δ 72.39 (-CH (CH$_2$O-)$_2$, δ 72.77 (-CH (CH$_2$OH).

Example 2: Synthesis of HPEG in Methylene Chloride

In a dry two neck round bottom flask fitted with dropping funnel 17 g (0.021 mol) pentaerithrytol ethoxylate and 10g (0.019 mol) polyethylene glycol diglycidyl ether was added in argon atmosphere. To this monomer mixture 300 ml of anhydrous methylene chloride was added by cannula. The monomer solution was kept at –10° C for 30 minutes. To the cold monomer solution 0.01 mol boron trifluoride solution in 10 ml anhydrous methylene chloride was added drop wise over a period of 1 hour. The polymerization was continued for 3 hours at –10° C and additional 16 hours at room temperature. The polymer was purified by repeated precipitation from petroleum ether and dried at vacuum. One part of the polymer was then dissolved in water and dialyzed against water to remove the un-reacted impurities. The dried polymer is nearly colorless gel like highly elastic material. The polymers readily dissolve in water and various organic solvents. Yield was 23 gm. Mn = 24.7, PDI = 1.87. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.47 (-CHCH$_2$OH-), δ 3.49 (-CHCH$_2$OH), δ 3.51 (-OCH$_2$-). ^{13}C NMR (100 MHz, DMSO-d_6) : δ 28.15 (C (CH$_2$O-)$_4$), δ 60.26 (-CH$_2$CH$_2$OH), δ 61.623 (-CHCH$_2$OH), δ 65.84 (C (CH$_2$O-)$_4$), δ 69.84 (-OCH$_2$CH$_2$-), δ 70.56 (-CH$_2$CH$_2$OH), δ 72.37 (-CHOH), δ 72.67 (-CH (CH$_2$O-)$_2$, δ 75.1 (-CH (CH$_2$OH).

Example 3: Synthesis of Tosylate-HPEG

2 g HPEG2 and 5.75g (30 mmol) p-toluene sulfonyl chloride was co-dissolved in 25 ml anhydrous methylene chloride under inert condition. The solution was placed in 0° C ice bath under argon atmosphere. 4.3 ml (30 mmol) of anhydrous triethylamine was added to the reaction flask. The reaction was continued 2 hours at 0° C and 16 hours at room temperature. The polymer was filtered and the polymer isolated by precipitating from ethyl ether. It was then re-dissolved in methylene chloride and precipitated from ethyl ether. This process was repeated 3 times. Yield 1.8 gm. ^{1}H NMR (400 MHz,

$CDCl_3$): δ 1.62 (-$OCH_2CH_2CH_2CH_2O$-), δ 2.34 (-$CH(CH_2-)_3$), δ 2.45 ($CH_3-C_6H_5$-), δ 3.41 (-OCH_2- in THF-THF homodiad), δ 3.46-3.52 (-OCH_2- in THF-EO heterodiad), δ 3.48-3.52 (-OCH_2- in EO-THF heterodiad), δ 3.60-3.64 (-OCH_2- in EO-EO homodiad), δ 4.16 (-CH_2O-SO_2-), δ 5.15 –$CHOSO_2$-), δ 7.3 (aromatic $CH_3C(CH)_2(CH)_2$, δ 7.8 (aromatic $(CH)_2$-C-SO_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.61 ($CH_3-C_6H_5$-), δ 26.51 (-$OCH_2CH_2CH_2CH_2O$-), δ 29.3 (C $(CH_2O-)_4$), δ 68.68 (-CH_2OSO_2), δ 69.21 (-$CHOSO_2$-), δ 69.43 (-C $(CH_2O-)_4$), δ 70.06 (-OCH_2CH_2- in EO-THF heterodiad), δ 70.36 (-OCH_2CH_2- in THF-THF homodiad), δ 70.60 (-OCH_2CH_2- in EO-EO homodiad), δ 70.94 (-OCH_2CH_2- in THF-EO heterodiad), δ 72.72 (-CH $(CH_2O-)_2$, δ 127.9 (aromatic $(CH)_2$-C-SO_2-), δ 129.8 (aromatic (CH_3-C-(CH)2(CH)2), δ 144.7 (aromatic (CH_3-C-$(CH)_2$ $(CH)_2$).

Example 4: Synthesis Of HPEG-B-Poly (2-Ethyl 2-Oxazoline) (HPPEOX)

0.75g of HPEG-tosylate was dissolved in 10 ml of anhydrous acetonitrile. 5 ml of distilled 2-ethyl 2-oxazoline monomer was added to it. It was then kept at reflux temperature for 5 days. The polymerization was terminated by 0.1 N methanolic KOH. The polymer was dissolved in water and dialyzed and isolated by freeze-drying. Yield 2.5 gm. Mn = 67.3K, PDI = 1.78. ^1H NMR (400 MHz, D_2O): δ 1.49 (CH_3-CH_2-CO-), δ 1.78 (-$CH_2CH_2CH_2CH_2O$- and), δ1.99-2.10 (CH_3CH_2CO-), δ 2.30 (-$CH(CH_2-)_3$), δ 3.2-3.44 (-$CH_2CH_2NCOCH_2CH_3$-), δ 3.46 (-OCH_2- in THF-EO heterodiad), δ 3.52 (-OCH_2- in EO-THF heterodiad), δ 3.59 (-OCH_2- in EO-EO homodiad).

Example 5: Hydrolysis Of HPEG-B-Poly (2-Ethyl 2-Oxazoline) (BPLP4)

0.5 g HPEG-b-Poly (2-ethyl 2-oxazoline) was dissolved in 10 ml ethylene glycol/ethanol (1:1 v/v) co-solvent. 0.6 g NaOH was added to it. The mixture was stirred for 16 hours at 90° C. The polymer solution was mixed with water and purified by ultrafiltration using 30K NMWC cartridge. Mn = 49.3K, PDI = 1.54. ^1H NMR (400 MHz, D_2O): δ 1.78 (-$OCH_2CH_2CH_2CH_2O$- and), δ 2.33 (-$CH(CH_2-)_3$), δ 2.59-3.09 (-$NHCH_2CH_2$-), δ 3.58 (-OCH_2- in EO-EO homodiad).

Example 6: Synthesis Of Chloromethyl HPEG (HPEG-Cl):

2 g HPEG2 and 1g (5 mmol) 4-chloromethyl benzoyl chloride was co-dissolved in 25 ml anhydrous methylene chloride under inert condition. The solution was placed in

0 °C ice bath under argon atmosphere. 1 ml of anhydrous triethylamine (7 mmol) was added to the reaction flask. The reaction was continued 2 hours at 0 °C and 16 hours at room temperature. The polymer was isolated by precipitating from ethyl ether. It was then dissolved in water, filtered, and freeze dried to get HPG-Cl. Yield 1.9 g. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.62 (-$OCH_2C\underline{H}_2C\underline{H}_2CH_2O$-), δ 2.25 (-$C\underline{H}(CH_2O$-)$2CH_2OH$), δ 2.49 (-$C\underline{H}(CH_2O$-$)_3$), δ 3.2 (-$OC\underline{H}_2$- in THF-THF homodiad), δ 3.41 (-$OC\underline{H}_2$- in THF-EO heterodiad), δ 3.59 (-$OC\underline{H}_2$- in EO-THF heterodiad), δ 3.64 (-$OC\underline{H}_2$- in EO-EO homodiad), δ 3.73-3.76 (HO-$C\underline{H}_2$-), and –$C\underline{H}O(CH2O$-)), δ 3.82 (-$OC\underline{H}_2CH_2OCO$-) δ 4.47 (-$C\underline{H}_2OCOC_6H_5$-), δ 4.52 (-$C\underline{H}OCOC_6H_5$-), δ 4.61-4.62 ($ClC\underline{H}_2C_6H_5$-), δ 7.47-7.49 (aromatic $ClCH_2(C\underline{H})2(CH)2$-), δ 8.05-8.08 (aromatic ((C$\underline{H}$)2COO)). ^{13}C NMR (100 MHz, $CDCl_3$) : δ 26.44 (-$OCH_2C\underline{H}_2C\underline{H}_2CH_2O$-), δ 45.31 ($ClC\underline{H}_2C_6H_5$-), δ 64.2 (-$C\underline{H}_2OH$), δ 69.1 (C ($C\underline{H}_2O$-)$_4$), δ 69.98 (-$OC\underline{H}_2CH_2$- in EO-THF heterodiad), δ 70.29 (-$OC\underline{H}_2CH_2$- in THF-THF homodiad), δ 70.54 (-$OC\underline{H}_2CH_2$- in EO-EO homodiad), δ 70.88 (-$OC\underline{H}_2CH_2$- in THF-EO heterodiad), δ 71.16 ((-$C\underline{H}_2CH_2OH$), δ 72.15 (-$C\underline{H}OH$), δ 128.4 (aromatic $ClCH_2C(C\underline{H})2$), δ 130.0-130.4 (aromatic $OCOC(C\underline{H})2$-), δ 132.4 (aromatic $(CH)2C\underline{C}COO$-), δ 142.2 (aromatic $ClCH2C(CH)2$-), δ 168.9 (aromatic -$C_6H_5C\underline{O}O$-).

Example 7: Synthesis of HPEG-B-DMEMA

0.25g HPEG-Cl and 2 ml of 2-(dimethylamino) ethyl methacrylate was dissolve in 10 ml of anhydrous THF. 15 mg (0.1 mmol) copper bromide and 45 µl PMDETA was added to it under argon atmosphere. The mixture was placed in 60 °C oil bath for 8 hours. The polymer was isolated by precipitating from ethyl ether. The polymer was purified by washing several times with diethyl ether. Yield 1 g. ^{1}H NMR (400 MHz, $CDCl_3$) (Fig 2) : δ 0.85-1.02 (from DMEMA, -$CH_2C(C\underline{H}_3)COO$-), δ 1.41-1.62 (from DMEMA, -$C\underline{H}_2C(CH_3)COO$- and -$OCH_2C\underline{H}_2C\underline{H}_2CH_2O$-), δ 1.81 (from DMEMA, -$N(C\underline{H}_3)_2$-), δ 1.901 (from DMEMA, -$OCH_2C\underline{H}_2N$-), δ 2.49 (-$C\underline{H}(CH_2O$-$)_3$), δ 3.41 (-$OC\underline{H}_2$- in THF-EO heterodiad), δ 3.59 (-$OC\underline{H}_2CH_2$- in DMEMA and EO-THF heterodiad), δ 3.64 (-$OC\underline{H}_2$- in EO-EO homodiad), δ 3.77 (HO-$C\underline{H}_2$-), and –$C\underline{H}O(CH_2O$-)), δ 3.83 (-$OC\underline{H}_2CH_2OCO$-) δ 4.47 (-$C\underline{H}_2OCOC_6H_5$-), δ 4.62 ($ClC\underline{H}_2C_6H_5$-), δ 7.45-7.52 (aromatic $ClCH_2(C\underline{H})2(CH)2$-), δ 8.03-8.14 (aromatic ((C$\underline{H}$)$_2$COO)).

Example 8: Synthesis Of HPEG-B-Poly (2-Methyl 2-Oxazoline) (HPPMOX1)

1 g of HPEG-Cl were dissolved in 10 ml distilled benzonitrile. 10 ml of distilled 2-methyl 2-oxazoline was added under anhydrous condition. 0.2 g potassium iodide was added. The mixture was placed in an oil bath at 90 °C. After 48 hours polymerization was terminated by 0.1 N methanolic KOH and the polymer was isolated by precipitating from acetone. The polymer was purified by dissolving in water and dialyzed against water. The GPC profile of polymer shown in Fig 1. Yield 9 g. ^{1}H NMR (400 MHz, D_2O) (Fig 3) : δ 1.82 (-$OCH_2C\underline{H}_2C\underline{H}_2CH_2O$-), δ 1.92-1.99 ($C\underline{H}_3CON$-), δ 2.15 (-$C\underline{H}(CH_2O$-)$2CH_2OH$), δ 2.59 (-$C\underline{H}(CH_2O$-$)_3$), δ 3.13 (-$OC\underline{H}_2$- in THF-THF homodiad), δ 3.4 (-$NC\underline{H}_2CH_2$-), δ 3.43 (-$OC\underline{H}_2$- in EO-EO homodiad), δ 3.49 (HO-$C\underline{H}_2$-), and –$C\underline{H}O(CH_2O$-)), δ 3.55 (-$OC\underline{H}_2CH_2OCO$-), δ 7.95 (aromatic -$NCH_2(C\underline{H})_2(CH)_2$-), δ 8.23 (aromatic (($C\underline{H})_2COO$)).

Example 9: Hydrolysis Of HPEG-B-Poly (2-Methyl 2-Oxazoline) (BPLP1)

1 g HPEG-b-Poly (2-methyl 2-oxazoline) was dissolved in 10 ml ethylene glycol/ethanol (1:1 v/v) co-solvent. 1.2 gm NaOH was added. The mixture was stirred for 16 hours at 90 °C. The polymer solution was mixed with water and purified by ultrafiltration using 30K NMWC cartridge. The GPC profile is shown in Fig 1. ^{1}H NMR (400 MHz, D_2O): δ 1.82 (-$OCH_2C\underline{H}_2C\underline{H}_2CH_2O$-), δ 2.02 (-$C\underline{H}(CH_2O$-)$2CH_2OH$), δ 2.60 (-$C\underline{H}(CH_2O$-$)_3$), δ 2.62-2.78 ($NC\underline{H}_2C\underline{H}_2$-), δ 3.29 (-$OC\underline{H}_2$- in THF-EO heterodiad), δ 3.39 (-$OC\underline{H}_2$- in EO-THF heterodiad), δ 3.41 (-$OC\underline{H}_2$- in EO-EO homodiad), δ 3.52 (HO-$C\underline{H}_2$-), and –$C\underline{H}O$(CH2O-)), δ 3.58 (-$OC\underline{H}_2CH_2OCO$-), δ 7.28-7.36 (aromatic $NCH_2(C\underline{H})_2(CH)_2$-), δ 7.7-7.8 (aromatic (($C\underline{H})_2COO$)).

Example 10: Synthesis of HPGG

In a typical synthesis 21 g (0.026 mol) pentaerithrytol ethoxylate (or polycaprolactone triol) and 4.3 g (0.021 mol) glycerol diglycidyl ether was added in argon atmosphere in a dry two neck round bottom flask fitted with dropping funnel. To this monomer mixture 300 ml of anhydrous tetrahydrofuran (THF) was added by cannula. The monomer solution was kept at –10 °C for 30 minutes. To the cold monomer solution 0.02 mol boron trifluoride solution in 10 ml anhydrous THF was added drop wise over a period of 1 hour. Two different catalyst concentration 0.02 mol and 0.01 mol BF_3 was used to get two different molecular weight polymers. All the reagents were used without

further purification. The polymerization was continued for additional 3 hours at –10 °C and at room temperature for 16 hours. The polymer was purified by repeated precipitation from petroleum ether and dried under vacuum. One part of the polymer was then dissolved in water and dialyzed against water to remove the un-reacted impurities. The dried polymer is nearly colorless gel like highly elastic material. The polymers readily dissolve in water and various organic solvents. Yield was 23 gm. In case of polymer prepared by polycaprolactone triol was not soluble in water. This polymer completely dissolves in THF or methylene chloride (characterization data of this polymer HPCG is not presented here). Mn of HPGG2 is 29.8K (PDI 2.20). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.51 (-OCH$_2$CH$_2$CH$_2$CH$_2$O-), δ 3.31-3.33 (-OCH$_2$- in THF-THF homodiad), δ 3.41 -3.43 (-OCH$_2$- in THF-EO heterodiad), δ 3.48 (-OCH$_2$- in EO-THF heterodiad), δ 3.51 (-OCH$_2$- in EO-EO homodiad), δ 3.85 (HO-CH$_2$- and –CHO(CH2OH)). ^{13}C NMR (100 MHz, DMSO-d_6) : δ 26.08 (-OCH$_2$CH$_2$CH$_2$CH$_2$O-), δ 44.38 (C (CH$_2$O-)$_4$), δ 45.34 (-C (CH2OH)$_3$), δ 60.21 (-CH$_2$CH$_2$OH), δ 60.62, δ 68.5 (C (CH$_2$O-)$_4$), δ 69.48 (-OCH$_2$CH$_2$- in EO-THF heterodiad), δ 69.55 (-OCH$_2$CH$_2$- in THF-THF homodiad), δ 69.78 (-OCH$_2$CH$_2$- in EO-EO homodiad), δ 70.10 (-OCH$_2$CH$_2$- in THF-EO heterodiad), δ 70.50 ((-CH$_2$CH$_2$OH), δ 71.49 (-CHOH), δ 72.17 (-CH (CH$_2$O-)$_2$, δ 72.34 (-CH (CH$_2$OH), δ 78.06 (-CHO(CH$_2$O-)$_2$).

Example 11: Synthesis of Chloromethyl HPGG (HPGG-Cl)

2 g HPEGG and 1 g (5 mmol) 4-chloromethyl benzoyl chloride was co-dissolved in 25 ml anhydrous methylene chloride under inert condition. The solution was placed in 0 °C ice bath under argon atmosphere. 1 ml of anhydrous triethylamine (7 mmol) was added to the reaction flask. The reaction was continued 2 hours at 0 °C and 16 hours at room temperature. The polymer was isolated by precipitating from ethyl ether. It was then dissolved in water, filtered and freeze dried to get HPEG-Cl. Yield 1.9 g. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.51 (-OCH$_2$CH$_2$CH$_2$CH$_2$O-), δ 2.25 (-CH(CH$_2$O-)2CH$_2$OH), δ 2.49 (-CH(CH$_2$O-)$_3$), δ 3.3 (-OCH$_2$- in THF-THF homodiad), δ 3.43 (-OCH$_2$- in THF-EO heterodiad), δ 3.59 (-OCH$_2$- in EO-THF heterodiad), δ 3.64 (-OCH$_2$- in EO-EO homodiad), δ 3.73-3.76 (HO-CH$_2$-), and –CHO(CH2O-)), δ 3.82 (-OCH$_2$CH$_2$OCO-) δ 4.47 (-CH$_2$OCOC$_6$H$_5$-), δ 4.52 (-CHOCOC$_6$H$_5$-), δ 4.61-4.62 (ClCH$_2$C$_6$H$_5$-), δ 7.47-7.49 (aromatic ClCH$_2$(CH)2(CH)2-), δ 8.05-8.08 (aromatic ((CH)2COO)). ^{13}C NMR (100 MHz, $CDCl_3$) : δ 26.44 (-OCH$_2$CH$_2$CH$_2$CH$_2$O-), δ 45.31 (ClCH$_2$C$_6$H$_5$-), δ 46.64 (C

(CH_2O-)$_4$), δ 69.1 (C ($\underline{C}H_2O$-)$_4$), δ 69.98 (-O$\underline{C}H_2CH_2$- in EO-THF heterodiad), δ 70.29 (-O$\underline{C}H_2CH_2$- in THF-THF homodiad), δ 70.54 (-O$\underline{C}H_2CH_2$- in EO-EO homodiad), δ 70.88 (-O$\underline{C}H_2CH_2$- in THF-EO heterodiad), δ 71.16 ((-$\underline{C}H_2CH_2OH$), δ 72.15 (-$\underline{C}HOH$), δ 128.4 (aromatic $ClCH_2C(\underline{C}H)_2$), δ 130.0-130.4 (aromatic $OCOC(\underline{C}H)_2$-), δ132.4 (aromatic $(CH)_2\underline{C}COO$-), δ 142.2 (aromatic $ClCH_2\underline{C}(CH)_2$-), δ 168.99 (aromatic -$C_6H_5\underline{C}OO$-).

Example 12: Synthesis of HPGG-b-Poly (2-methyl 2-Oxazoline) (HPGMOX1)

1 g of HPEGG-Cl were dissolved in 10 ml distilled benzonitrile. 10 ml of distilled 2-methyl 2-oxazoline was added under anhydrous condition. 0.2 g potassium iodide was added. The mixture was placed in an oil bath at 90° C. After 48 hours polymerization was terminated by 0.1 N methanolic KOH and the polymer was isolated by precipitating from acetone. The polymer was purified by dissolving in water and dialyzed against water. Yield 2.5 g. Mn = 46.2K, PDI = 1.93. ^{1}H NMR (400 MHz,$CDCl_3$): δ 1.19-1.23 ($C\underline{H}_3$-CH_2-CO-), δ 1.98 ($C\underline{H}_3$CON-), δ 2.01 (-$C\underline{H}(CH_2O$-)$2CH_2OH$), δ2.11-2.14 ($CH_3C\underline{H}_2CO$-), δ 2.90 (-$C\underline{H}(CH_2O$-)$_3$), δ 3.46 (-$C\underline{H}_2C\underline{H}_2NCOCH_2CH_3$-), δ 3.50 (-$OC\underline{H}_2$- in EO-EO homodiad), δ 7.35 (aromatic -NCH2($C\underline{H}$)$_2$(CH)$_2$-), δ7.98-8.04 (aromatic (($C\underline{H}$)$_2$COO)

Example 13: Hydrolysis of HPGG-b-Poly (2-methyl 2-Oxazoline) (HGLP6)

1 g HPGPMOX was dissolved in 10 ml ethylene glycol/ethanol (1:1 v/v) co-solvent. 1.2 g NaOH was added. The mixture was stirred for 6 hours at 90 °C. The polymer solution was mixed with water and purified by ultrafiltration using 30K NMWC cartridge. Mn = 39.4K, PDI = 1.74. ^{1}H NMR (400 MHz, D_2O) : δ 2.01 (-$C\underline{H}(CH_2O$-)$2CH_2OH$), δ 2.62-2.78 ($NC\underline{H}_2C\underline{H}_2$-), δ 2.90 (-$C\underline{H}(CH_2O$-)$_3$), δ 3.41 (-$OC\underline{H}_2$- in EO-EO homodiad), δ 3.52 (-O-$C\underline{H}_2$-), and –$C\underline{H}O$(CH2O-)), δ 7.36 (aromatic $NCH_2(C\underline{H})_2(CH)_2$-), δ 7.98 (aromatic (($C\underline{H}$)$_2$COO)).

Example 14: Synthesis of HPGG-b-Lactide (HPGLA)

2 g dl-lactide and 0.5 gm HPGG-Cl was added in a preheated dry 25 ml reaction flask. 0.025 mmol tin ethyl hexanoate with 10 ml anhydrous toluene was added to the reaction flask. The reaction flask was then heated 16 hours at 130 °C under inert atmosphere. The polymer formed was dissolved in 10 ml chloroform and precipitated in

diethyl ether in presence of methanol. The polymer was dried in vacuum and characterized by 13C and 1H NMR. Yield is 1.5 g. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.47-COCH(C$\underline{H}_3$)O-), δ 3.43-3.47 (-OC$\underline{H}_2$- in THF-EO heterodiad), δ 3.50 (-OC$\underline{H}_2$- in EO-EO homodiad), δ 4.2 (-OC$\underline{H}$O- in EO-THF heterodiad), δ 4.60 (ClC$\underline{H}_2$C$_6$H$_5$-), δ 5.19-COC$\underline{H}$(CH$_3$)O-), δ 7.48 (aromatic ClCH$_2$(C$\underline{H}$)2(CH)2-), δ 8.1 (aromatic ((C$\underline{H}$)2COO)). ^{13}C NMR (100 MHz, DMSO-d_6) : δ 16.4 (-OCCH(C$\underline{H}_3$)O-), δ 26.06 (-OCH$_2$C$\underline{H}_2$C$\underline{H}_2$CH$_2$O-), δ 28.9 (C ($\underline{C}$H$_2$O-)$_4$), δ 68.6 (-OC$\underline{C}$H(CH3)O-), δ 68.8 (C ($\underline{C}$H$_2$O-)$_4$), δ 67.9 (-O$\underline{C}$H$_2$CH$_2$- in EO-THF heterodiad), δ 68.6 (-O$\underline{C}$H$_2$CH$_2$- in EO-EO homodiad), δ 69.7 (-O$\underline{C}$H$_2$CH$_2$- in THF-EO heterodiad), δ 72.1 (-$\underline{C}$H (CH$_2$O-)$_2$, δ 168.9-169.1 (-O$\underline{C}$OCH(CH$_3$)-).

Example 15: Synthesis of HPGLA-b-Poly (2-methyl 2-Oxazoline) (HPGLMOX)

1 g of HPGLA-Cl were dissolved in 10 ml distilled benzonitrile. 10 ml of distilled 2-methyl 2-oxazoline was added under anhydrous condition. 0.2 g potassium iodide was added. The mixture was placed in an oil bath at 90 °C. After 48 hours, polymerization was terminated by 0.1 N methanolic KOH and the polymer was isolated by precipitating from acetone. The polymer was purified by dissolving in water and dialyzed against water. Yield 2.5 g , PDI = 1.73.

^{1}H NMR (400 MHz, CDCl$_3$): δ 1.40-1.42-COCH(C$\underline{H}_3$)O-) and (C$\underline{H}_3$-CH$_2$-CO-), δ1.99-2.10 (CH$_3$C$\underline{H}_2$CO-), δ 2.30 (-C$\underline{H}$(CH$_2$-)$_3$), δ 3.4 (-NC$\underline{H}_2$CH$_2$-), δ 3.47-3.55 (-OC$\underline{H}_2$- in EO-EO homodiad), δ 4.1 (-C$\underline{H}_2$OCOC$_6$H$_5$-), δ 4.3 (-C$\underline{H}$OCOC$_6$H$_5$-), δ 5.19 -COC$\underline{H}$(CH$_3$)O-), δ 7.5-7.66 (aromatic NCH$_2$(C$\underline{H}$)$_2$(CH)$_2$-), δ 8.45 (aromatic ((C$\underline{H}$)$_2$COO)).

Example 16: Synthesis of HPGG-succinimide ester (HPGG-NHS)

5 g of HPGG was dissolved in 60 ml of toluene and placed in a two neck round bottom flask. The flask was connected with Dean-stark apparatus fitted with a condenser. The toluene solution of HPGG was refluxed to make dry HPGG toluene solution. After cooling to room temperature, 15 ml of phosgene solution (30 mmol) in toluene was added, and the resulting solution was stirred overnight. The chlorinated HPGG solution was evaporated by rotary evaporator and immediately re-dissolved with 30 ml anhydrous toluene. The toluene was removed by rotary evaporator and again re-dissolved in 20 ml dry toluene. 10 ml of anhydrous methylene chloride was added with 1.15 g (0.01 mol) of

N-hydroxysuccinimide. The reaction mixture was stirred vigorously with a magnetic stirrer and then placed at 0 °C water bath. To the reaction mixture, 1.4 ml (0.01 mol) cold anhydrous triethylamine was added dropwise. The reaction was stirred at 0° C for 2 hours, and then 16 hours at room temperature. 10 ml of toluene was added to the reaction mixture and placed in an ice-bath for 30 minutes to maximize the triethyl amine salt precipitation. The reaction mixture was filtered and the product solution was concentrated by rotary evaporator. The product was isolated by precipitation from cold ether. The polymer was dissolved in hot 2-propanol. After cooling the 2-propanol solution polymer precipitated and it was then isolated and dried under vacuum. Yield was 4 g.

Example 17: Synthesis of HPGG-g-BPEI (HPGBPEI)

15 g of branched polyethyleneimine (BPEI) (MW 10K) was dissolved in 300 ml anhydrous methylene chloride. 1 g of HPGG-NHS was dissolved in 30 ml anhydrous methylene chloride separately. 1 ml of N,N-diisopropylethyl amine was added to the BPEI solution. To this solution HPGG-NHS solution was added dropwise under inert conditions using a dropping funnel over a period of 30 minutes. The reaction was continued for 16 hours at room temperature. After the reaction, methylene chloride was evaporated and the polymer mixture was dissolved in water and filtered. The polymer was purified by ultrafiltration using a 30K NMWC cartridge. The polymer was isolated by lyophilization. Yield was 12 g. Mn =40.2K (PDI = 2.04), ^{1}H NMR (400 MHz, D_2O): δ 2.58-3.01 (NCH_2CH_2N-), δ 3.57 (-OCH_2- in EO-EO homodiad), δ 7.96-7.98 ($OCONHCH_2$-).

Example 18: Synthesis of Hyperbranched polyglycidol (HPG)

In a dry two neck round bottom flask fitted with a dropping funnel, 200 ml of anhydrous methylene chloride was cannulated under argon atmosphere. 0.04 mol boron trifluoride etherate was added to the reaction flask. The reaction flask was placed in –10° C for 30 minutes. In an additional round bottom flask, previously dried, 10 g glycerol (0.11 mol) and 20 gm glycidol (0.27 mol) was added with 100 ml anhydrous methylene chloride. The monomer mixture was kept at –10 °C for 30 minutes, and then transferred to the dropping funnel connected to the reaction flask. The monomer solution was then added dropwise to the initiator solution over a period of 1 hour. The reaction was continued at –10 °C for 3 hours. The polymer separated from methylene chloride.

The polymer was separated and dissolved methanol/DMF mixture. The polymer was then dialyzed against water using a 1K molecular weight cut off dialysis bag (Spectra/Por). The dialyzed product was isolated by lyophilization.

Example 19: Synthesis of Hyperbranched polyester (HPET)

0.75 g of HPG, 18 g (0.13 mol) of bis-MPA and 0.5 wt % based on bis-MPA, 90 mg p-toluene sulfonic acid was charged in a two neck round bottom flask fitted with a Dean-Stark apparatus and was placed in an oil bath at 150 °C. The reaction was continued for 16 hours, and after cooling to room temperature it was dissolved in THF. The polymer was precipitated in diethyl ether and dried at vacuum for 48 hours.

Example 20: Synthesis of HPET-succinimide ester (HPET-NHS);

5 g of HPET was dissolved in 30 ml of toluene and placed in a two neck round bottom flask. The flask was connected to a Dean-stark apparatus fitted with a condenser. The toluene solution of HPPEG was refluxed to make a moisture free HPPEG toluene solution. After cooling down to room temperature, 20 ml of phosgene solution (30 mmol) in toluene was added, and the solution was stirred overnight. The chlorinated HPPEG solution was evaporated by rotary evaporator and immediately dissolved in 30 ml anhydrous toluene. The toluene was removed by rotary evaporator and again re-dissolved in 20 ml dry toluene. 10 ml of anhydrous methylene chloride was added with 3 g (0.03 mol) of N-hydroxy succinimide. The reaction mixture was stirred vigorously with magnetic stirrer and then placed in a 0 °C water bath. To the reaction mixture 4.2 ml (0.03 mol) cold anhydrous triethylamine was added dropwise. The reaction was stirred for 2 hours at 0° C, and then for 16 hours at room temperature. 10 ml of toluene was added to the reaction mixture and placed in an ice-bath for 30 minutes. to maximize the triethyl amine salt precipitation. The reaction mixture was filtered and the product solution was concentrated by rotary evaporator. The product was isolated by precipitation from cold ether. The polymer was dissolved in methylene chloride and precipitated from ether. This process was repeated twice and the polymer was then dried under vacuum. Yield was 4.5 g.

Example 21: Synthesis of HPET-g-BPEI (HPEBPEI)

15 g of branched polyethyleneimine (BPEI) (MW 10K) was dissolved in 300 ml anhydrous methylene chloride. 1 g of HPET-NHS was dissolved in 30 ml anhydrous

methylene chloride separately. 1 ml of N,N-diisopropylethyl amine was added to the BPEI solution. To this solution HPET-NHS solution was added dropwise under inert conditions using a dropping funnel over a period of 30 minutes. The reaction was continued for 16 hours at room temperature. After the reaction, methylene chloride was evaporated and the polymer mixture was dissolved in water and filtered. The polymer was purified by ultrafiltration using a 30K NMWC cartridge. The polymer was isolated by lyophilization. Yield was 9 g.

Example 22: Cytotoxicity

Cells were plated at a density of 10,000 cells/well of 96 well plates in 150 µl of medium 24 hours before the actual experiment. Before the addition of polymer solution, medium were replaced by fresh 150 µl of serum containing media. The 50 µl polymer solution were then added to each well. The total volume did not exceed 200 µl/ well and the plates were incubated for 48 hours at 37°C. Cytotoxicity assays (n = 4 experiments) were performed using standard CytoTox96® non-radioactive cytotoxicity assay (Promega, Madison, WI), i.e., a colorimetric method that quantities lactate dehydrogenase (LDH), a stable cytosolic enzyme that is released upon cell lysis. Released LDH in culture supernatants were measured using a 30 minutes coupled enzymatic assay, which results in the conversion of tetrazolium salt into a red formazan product. The absorbance of the colored product formed was proportional to the number of lysed cells. The absorbance at 490 nm was measured using a 96 well ELISA plate reader. The background absorbance was minimized using phenol red free cell growth media. Results are shown in Fig. 5. In particular, the results showed that BPLP polymers had a low toxicity when compared to LPEI.

Fig. 8 shows cytotoxicity profiles of linear polyethyleneimine (LPEI), branched polyethyleneimine (BPEI) and hyperbranched block copolymers HPGBPEI, HGPL6, HGPL7 in COS-1 cell culture after 48 hours incubation.

Example 23: Transfection

COS1 cells were seeded at a density of 35,000 cells/well in 24-well plate, 24 hours prior to transfection. Before transfection, media was removed and replaced by fresh media containing 10% FBS. Cells were treated with complexes made using 1 mg of pCMV-Luc for 24 hours at 37° C. The concentration of polymers varied to get different polymer/DNA ratios. After 24 hours, the growth medium was removed, cells were rinsed

with PBS and 100 µl of reporter lysis buffer (Promega) was added. The cells were kept at –80 °C for 1 hour and then equilibrated to room temperature. Luciferase expression was measured by a luminescence assay. 20 µl of the lysate was dispensed into a well of 96-well plate and luciferase activity was integrated over 10 s with 2 s measurement delay using Microlumat LB96P (EG&G Berthold) with automatic injection of 100 µl of luciferase assay reagent (Promega). The protein content was determined by BCA assay (Pierce, Rockford, IL). Results were expressed as relative light units per mg of cellular protein. Results are summarized in Fig. 6, which shows at higher concentrations, the BPLP polymers show higher transfection efficiency than LPEI.

Fig. 9A is a graph that shows Luciferase expression in COS 1 cells after a 48 hours transfection in serum-containing mediun. In particular, it shows transfection efficiency of LPEI, HGPL6, HGPL7, HPGBPEI and BPEI:polymer complex with plasmid was prepared by mixing 1 mg of pCMV-luc with various polymer amounts (shown in x-axis).

Fig. 9B is a graph that shows percent inhibition of Luciferase expression in Gli36 cells after a 48 hour transfection of siRNA vector in serum-containing medium. In particular, inhibition in the presence control empty vector (EV) LPEI-EV, HGPL6-EV and Si-RNA against luciferase mRNA vector (Luc) HGPL6-Luc, LPEI-Luc. The polymer complex with plasmid was prepared by mixing 1 µg of pCMV-luc with various polymer amounts (shown in x-axis).

Example 24: Synthesis of Carboxy Derivatives of Hyperbranched Polymer HPG, (HPG-COOH)

5 g of HPG was dissolved in 100 ml water. 20 g (0.14 mol) of bromoacetic acid was dissolved in 50 ml water. To the bromoacetic acid solution 50 ml of 3M sodium hydroxide solution was added and cooled to room temperature. The HPG solution was then added to bromoacetic acid solution at room temperature. The reaction was kept stirring 24 hours at room temperature. The polymer was purified by ultrafiltration and isolated by freeze-drying. Yield was 4.8 g.

Example 25: Synthesis of HPG-NH_2

2 g of HPG-COOH was dissolved in 50 ml of 0.1M sodium bicarbonate buffer (pH 8.4). 500 mg of EDC was added, and stirring was continued for 30 minutes. 0.01 g

4-dimethylamino pyridine was added to it. 10 ml (15 mol) of ethylenediamine was dissolved in 30 ml of water. The ethylenediamine solution was then added dropwise to the activated HPG-COOH over a period of 30 minutes. The reaction mixture was stirred for 16 hours. The excess ethylenediamine was removed by rotary evaporator and the polymer was dissolved in water. The polymer solution was then dialyzed against water and then lyophilized to isolate the HPG-NH_2. Yield was 1.9 g.

Example 26: Synthesis of HPG-LYSINE

5.1 g (9.7 mmol) of boc-protected lysine was dissolved in 20 ml of anhydrous methylene chloride at 0 °C. To this solution, 1.2 ml (9.7 mmol) of trimethylacetyl chloride was added, and then 1.4 ml (10 mmol) of anhydrous triethyl amine was added. In a separate reaction flask, 5 g of hyperbranched polyethylene glycol (HPG) was dissolved in 20 ml anhydrous methylene chloride and 1.4 ml of triethylamine was added. The polymer solution was kept at 0 °C. To the acetyl derivative of lysine was added the HPG solution by needle under an inert atmosphere. The reaction mixture was stirred for 2 hours at 0 °C, and then for 16 hours at room temperature. The polymer was isolated by repeated precipitation from ethyl ether and dried in vacuum.

The polymer (2 g) was cleaved by dissolving in 50 ml de-protection mixture (44 ml TFA, 2.5 ml water, 1 ml TIS and 2.5 g DTT) at room temperature for 3 hours. After cleavage the polymer solution was dialyzed against water and isolated by lyophilization.

Example 27: Synthesis of HPG-NH2-CY3

The HPG-NH2 was modified with the Cy3 N-hydroxysuccinimide ester (Amersham-Pharmacia Biotech). HPG-NH2 1 mg was dissolved in 200 µl of 0.05 M $NaHCO_3$ pH 8.7 and added to the dried contents of Cy3-NHS modification vial (Amersham-Pharmacia). This amount of Cy3 dye, formulated by the manufacturer for antibody modification resulted in desirable modification yield. Three hours later, a HPG-NH-Cy3 probe was purified by two sequential spins on BioGel® P30 mini-columns (Bio-Rad) saturated with BSA and equilibrated with sterile PBS. The amount of Cy3 attached to HPG-NH2 was verified using a UV-Vis spectrophotometer at 552 nm. The free amino group either can be used for further modification or can be blocked by reacting with excess mPEG-NHS. The purified polymer size was determined using HPLC and Zetasizer®.

Example 28: Synthesis of HPG-NH2-PEPTIDE-DYE

The HPG-NH2 (1 mg) in 200 µl of 50 mM NaHCO3 was iodoacetylated by reaction with excess of iodoacetic anhydride (3.5 mg, 10 µmol) in 100 µl of DMF for 3 hours. A micro concentrator, 50-kDa cutoff (Amicon, Beverly, MA), was used to separate the product from excess reagents, byproducts, and solvents. The iodoacetylated HPG was then coupled to 3.2 mg (2 µmol) of a cleavable peptide having thiol end groups through a thiol specific reaction in 200 µl of acetonitrile and 200 µl of 0.1 M sodium acetate, pH 6.5, buffer for 3 hours. Excess peptide and byproducts were separated from peptide-HPG conjugate by P-10 BioGel® (Bio-Rad, Hercules, CA) size-exclusion chromatography. Monoactivated dye Cy3 (Amersham-Pharmacia, Piscataway, NJ), 1 µmol, was reacted to the N-termini of the peptide-HPG (0.5 mg) in 2 ml of 50 mM NaHCO3. The reaction mixture was incubated at room temperature for 1 hour, and products were purified using a 50 kDa cutoff Microcon® concentrator.

Example 29: Synthesis of HPG-NH2-DOTA

The HPG-NH2 was modified with the DOTA-NHS [1,4,7,10-tetraazacyclododecane-1, 4,7,10-tetraacetic acid mono (N-hydroxysuccinimidyl ester)]. HPGNH2 1 mg was dissolved in 200 µl of 0.05 M $NaHCO_3$ pH 8.7 and added to required DOTA-NHS. Three hours later, a HPG-NH-DOTA probe was purified by two sequential spins on BioGel® P30 mini-columns. The purified DOTA derivative was reacted with gadolinium chloride in citric acid solution (pH ~ 6) for 1 hour at room temperature and purified.

Example 30: RNAi Experiment

SiRNA expressing plasmid DNAs driven by H1 promoter were constructed from pSuper-retro-GFP (Oligoengine, Seattle, WA). Targeted siRNA sequences were designed against the pGL3 basic vector firefly luciferase with a loop sequence TTCAAGAGA that was flanked by transcription terminators composed of five thymidines. The duplex of the 60 base pairs was cloned in the vector linearized with HindIII and BglII. The control vector was used without having any insert. The obtained plasmid vector pH1-siLuc was propagated in *E. coli* DH5α, and purified using Megaprep® columns (Qiagen, Valencia, CA). Plasmid integrity was confirmed by gel electrophoresis in agarose. The presence of the correct siRNA insert was confirmed by digesting the vector using EcoRI and HindIII

and sequencing. DNA concentration and purity was determined by measuring absorbance at 260 nm and 280 nm.

pH1-siLuc and the control vector having no siLuc insert (empty vector) were used for RNAi delivery to cells. In transient transfection exoperiments, 40,000 Gli36 cells were seeded into six well plates 24 hours prior to transfection. In all transfections, a total of 2 μg of plasmid DNA per well was used. The complexes were made by mixing the plasmid DNA and with the appropriate polymer (either HPGBPEI or BPEI) in GHB. The complexes were incubated with cells for 24 hours with serum containing media, followed by replacing the medium. After 48 hours, the total number of cells in each well were counted and the cells were lysed using luciferase assay lysis buffer (Promega).

In a co-transfection experiment, the transfection and luminescence measurements were performed exactly as described above using a mixture of 1 μg of pGL3 control vector (Promega) and 1 μg pH1-siLuc or empty vector instead of pH1-siLuc.

The siRNA plasmid was delivered to glioblastoma cells using either HPGBPEI or BPEI (see Example 17). Results are summarized in Fig. 10A and 10B which show levels of silencing of luciferase expression was similar for both HPGBPEI or BPEI (silencing approximately 80 percent for both). In addition, the results indicate that HPGBPEI shows efficient knocking down of luciferase expression when both luciferase-encoding pCMC-Luc plasmid and Luc-siRNA plasmid were co-transfected in HeLa cells.

OTHER EMBODIMENTS

While Fig. 7 shows moieties bound to the outer shell of a hyperbranched polymer, the moieties can also be encapsulated within the core of the hyperbranched polymer.

Any of the polymers described herein can be activated toward addition reactions, for example, 2 + 4 cycloaddition reactions.

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.